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A SURVEY OF PURINE DERIVATIVES AS INDUCERS OF CHROMOSOME CHANGES

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I. MUTAGENIC EFFECT ON MICRO-ORGANISMS.

THAT mutations can be induced by caffeine and theophylline was shown by FRIES and KIHLMAN (1948). They found a considerably higher percentage of biochemical mutants in conidial cultures of the ascomycete *Ophiostoma multiannulatum* treated with these purine derivatives than in untreated material. Thus, after a 5-days treatment with 0,2 % caffeine, 0,8 % mutant conidia was obtained, while untreated conidial cultures were found to contain never more than 0,06 %. A 4-days treatment with 0,6 % theophylline resulted in 0,6 % mutant conidia.

FRIES (1950) showed that the increased percentage of mutants resulting from caffeine treatments cannot be explained merely as a result of a selection, through differential killing, of mutants already existing in the conidial culture.

Independently of the experiments of FRIES and KIHLMAN, WITKIN demonstrated that caffeine is able to induce mutations to phage resistance in the bacterium *Escherichia coli* (quoted from DEMEREC, 1949), results which have been confirmed recently by GEZELIUS and FRIES (1952). DEMEREC, BERTANI and FLINT (1951) have found that caffeine is able to induce back-mutations from streptomycin dependence to non-dependence in *E. coli*.

II. RADIOMIMETIC EFFECT ON HIGHER PLANTS.

1. INVESTIGATIONS ON ALLIUM CEPA.

Since mutagenic substances in general are able to induce structural chromosome changes, caffeine was also tested in cytological experiments. As a result of the experiments of GOSSELIN (1940) and DEYSSON (1948), *etc.*, it is known that caffeine (1) inhibits cell-wall formation after mitosis so that binuclear and later multinuclear cells are produced, and (2) induces bridge formation at anaphase.

In the investigations of KIHLMAN and LEVAN (1949), root tips of

common onion, *Allium cepa*, were used as experimental material. The experiments showed that solutions containing more than 0,04 % of caffeine suppress mitosis, a total suppression being obtained with 0,4 % caffeine. In concentrations stronger than 0,02 % caffeine, as well as theophylline and theobromine, suppresses cell-wall formation. The methylxanthines mentioned do not inhibit spindle function, *i. e.*, they do not induce full c-mitosis.

Of particular interest in this connection are the effects on the chromosomes themselves. Using caffeine in concentrations above 0,04 %, chromosome changes were observed within 1 hr. after commencement of the treatment. These changes were mostly of the type due to stickiness of the chromosome matrix, *i. e.*, clumping of the chromosomes at metaphase, as well as formation of chromatin bridges between separating chromosome arms at anaphase. Another change appearing soon after the beginning of the treatment is the so-called pseudo-chiasma phenomenon (LEVAN, 1949).

The formation of anaphase bridges after caffeine treatment, as observed by DEYSSON, was probably due to primary effects of the types just described. KIHLMAN and LEVAN found, however, that after caffeine treatments a secondary effect is also demonstrable. [The terms »primary» and »secondary» effect were originated by ALBERTI and POLITZER (1923). According to these workers, the »Primäreffekt» is exhibited by cells already in division at the time of treatment; while the »Sekundäreffekt» is observable in cells entering mitosis after the end of the »mitosenfreie Zwischenzeit», which follows the treatment. Secondary effects are thus induced in resting cells.] Secondary effects were studied in root tips which had been treated for 24 hours in solutions containing 0,1—0,4 % caffeine and afterwards allowed to recover in water, the recovery period being 1—7 days. The changes observed were of the usual type known from X-ray experiments and treatments with mustards, *e. g.*, fragmentations and chromosome bridges at anaphase. The bridges expose most of the dicentric chromatids which have arisen as a result of sister chromatid union in centric fragments at the point of breakage, or as a result of reciprocal translocation. After the recovery period, the chromosomes showed no sign of stickiness.

The results of KIHLMAN and LEVAN were later confirmed qualitatively by LOVELESS and REVELL (1949).

Thus it was found that caffeine, in addition to having a mutagenic effect on micro-organisms, in cytological experiments is able to induce chromosome changes of the same kind as are obtained after X-ray

irradiation, *i. e.*, it has a *radiomimetic* effect. The question now arises: Upon what chemical and physical properties of the caffeine molecule does its ability to induce chromosome changes depend? By testing the effects of a great number of compounds closely related to caffeine, it should perhaps be possible to answer this question. The following purines and related compounds were therefore synthesized: 1,7-dimethylhypoxanthine (DMH), 8-chlorocaffeine (CC), 8-methoxycaffeine (MOC), 8-ethoxycaffeine (EOC), 8-propyloxycaffeine (POC), 8-allyloxycaffeine (AOC), 8-methylthiocaffeine (MTC), 8-ethylthiocaffeine (ETC), 1,3,7-trimethyluric acid or 8-hydroxycaffeine (HOC), 1,3,7,9-tetramethyluric acid (TMU), 9-ethyl-1,3,7-trimethyluric acid (ETMU), 9-allyl-1,3,7-trimethyluric acid (ATMU), and 1,3,7,9-tetramethyl-4,5-dimethoxydihydrouric acid (TDDU). From other sources the following were obtained: hypoxanthine, xanthine, uric acid, adenine, guanine, theophylline, theobromine, 8-methylaminocaffeine (MAC), 1,3-diethylxanthine (DEX), 1,3,7-triethylxanthine (TEX), and 8-hydroxy-9-methyl-8,9-dihydrocaffeine (HMDC). The effects of these 24 compounds on the root-tip chromosomes of *Allium cepa* were thereupon tested. Detailed descriptions and discussions of the method used and the results obtained have recently been published (KIHLMAN, 1951, 1952). The main results are summarized below.

Toxicity. — The purine derivatives in general have a lethal effect only in high concentrations. If in methylated oxypurines, the methyl groups are substituted by ethyl or other alkyl groups with more carbon atoms in the chain, the toxicity is increased. Thus, DEX and TEX are considerably more toxic than the corresponding methylxanthines, *viz.*, theophylline and caffeine. This rule is valid not only for nitrogen-bound alkyl groups but for alkoxy groups as well. The toxicity is further correlated with the temperature during the period of treatment; the higher the temperature, the greater being the toxicity.

Inhibition of spindle function. — In general purine derivatives do not induce full c-mitosis. Exceptions are the ethylxanthines and adenine. The former compounds obey the rule formulated by LEVAN and ÖSTERGREN (1943), *viz.*, that the lower the water solubility of the substances, the lower their threshold concentrations for c-mitotic action. (The decisive factor in c-mitosis, however, should not be the water solubility of the substances but their relative lipoid solubility, which was assumed to be inversely proportional to the water solubility.) However, of the purines tested, adenine has both the most pronounced c-mitotic action and the lowest relative lipoid solubility. It would seem, therefore,

that the c-mitotic action of adenine, like that of colchicine, may be due to a more specific reaction than that connected with relative lipoid solubility.

The failure of cell-wall formation. — The ability to suppress cell-wall formation seems to be a property common to all N-alkylated oxypurines. It is, however, seldom so pronounced as after treatments with caffeine. A strong effect is also observed after treatments with MTC. As already mentioned, the suppression of cell-wall formation results in the origin of bi- and multinuclear cells. In such cells the nuclei may fuse during mitosis producing cells with a high number of chromosomes. Polyploid cells may thus arise as a result of two different mechanisms after treatment with purine derivatives, namely (1) c-mitosis (adenine, ethylxanthines) and (2) suppression of cell-wall formation (N-alkylated oxypurines).

Effects on chromosomes. — In general, the chromosome changes observed are of the same type as those already described in connection with the caffeine experiments, though from a quantitative point of view the purines tested behaved very differently. Some of them were totally or practically inactive, while others had an activity many times greater than that of caffeine. The most pronounced stickiness effect was obtained after treatments with the 8-ethers and 8-thioethers of caffeine, but also CC and TMU were active in this respect. Pseudo-chiasmata were particularly common after treatments with MAC.

In order to compare the chromosome-breaking abilities of the purines, their effects were calculated on a molar basis. The purines were classified as (1) very active, (2) active, (3) slightly active, and (4) inactive. The »very active» type comprised all the 8-substituted caffeine derivatives tested, HOC excepted. A marked effect was also obtained with TMU. In the *Allium* experiments, all the non-alkylated oxypurines were inactive, as were also DMH, the ethylxanthines, HOC, TDDU, and HMDC. Adenine, in high concentrations (40—20mmol/l.), possessed a rather marked chromosome-breaking ability; however, calculated on a molar basis, its effect is slight compared with that of the 8-substituted caffeine derivatives. After adenine treatments, such disturbances as »attached fragments» and »chromosome erosion» (LEVAN and TJIO, 1948) were unusually frequent.

The following relations were found between the radiomimetic effect of purines and their chemical constitution:

(1) The radiomimetic activity of oxypurines increases with increasing N-methylation.

(2) The activity of methylated oxypurines diminishes or is completely lost if methyl groups are substituted with groups possessing longer carbon chains. (In this respect the radiomimetic effect is inversely proportional to the toxicity.)

(3) In caffeine, substitution of the hydrogen atom at C_8 with an electro-negative substituent, generally results in an increase in activity.

(4) The inability of HOC to induce chromosome changes indicates that the introduction of an acid, ionizable group in the molecule destroys the radiomimetic activity.

Further, it seems as if the double bond adjacent to C_8 or the 4—5 double bond should be of significance with regard to radiomimetic activity.

Chromosome breaks and rearrangements, as well as stickiness and pseudo-chiasmata, are induced by purines only above a certain threshold concentration which differs for different purines. This threshold is considerably higher (up to ten times) for the former type of changes than for the latter. Pseudo-chiasmata may be induced by more dilute solutions than may stickiness, and the threshold is thus lowest for this type of chromosome change.

It was also found that the active purine derivatives did not behave similarly in the experiments. Two types could be distinguished: The first is represented by ETC and the 8-ethers of caffeine, with the exception of MOC. These purines act rapidly, so that after a few hours most of the treated cells showed chromosome breaks. The effect increases in proportion to the concentration and is independent of the mitotic activity during the period of treatment. This mode of action is clearly demonstrated in the effect of EOC. To the EOC group belong such purines as have the same mode of action.

With the other type of active purine derivatives, chromosome breaks are obtained in a large number of cells only after a long period of treatment (about two days), and in concentrations sufficiently low to permit a certain amount of mitotic activity during treatment. After treatment with concentrations suppressing mitosis completely, the percentage of abnormal cells is always low. Thus, it is evident that the percentage of abnormal cells, obtained after treatment with purines of this group (the TMU group), is dependent on the mitotic activity during the treatment-period; the more mitoses occurring during the treatment, the greater the number of abnormal cells. When the effect of this group of purines is expressed in terms of the number of breaks per abnormal

cell, however, it shows the same dose proportionality as does the effect of the purines belonging to the EOC group.

The effect of purine derivatives on chromosomes is more or less independent of the *pH* of the treatment solution. On the other hand, the *temperature* during the period of treatment is of great significance. It is of interest to note that the temperature influences the effect of EOC and TMU in two different ways. With EOC, the strongest effect is obtained when the temperature during treatment is around 10° C. The effect at 30° C is only three quarters of that at 10°C. With TMU, however, only a low number of abnormal cells is obtained at 10° C, this purine being most effective at temperatures around 20° C. The explanation is that at 10° C the mitotic activity is low, and since the radiomimetic effect of TMU, if expressed in percentage of abnormal cells, is dependent on mitotic activity, it must also be low.

2. INVESTIGATIONS ON PISUM SATIVUM.

Some of the purine derivatives were also tested in experiments with *Pisum sativum* (KIHLMAN, 1952). The same types of chromosome changes and the same quantitative differences in activity between the purines, as previously observed in the *Allium* experiments, were found. The root-tip chromosomes of *Pisum*, however, are much less susceptible to the radiomimetic effects of the purines than are those of *Allium*, this rule applying to the primary as well as to the secondary effect. The characteristic difference between EOC and TMU as regards their mode of action did not appear so clear-cut in the *Pisum* experiments as in *Allium*, the reason being that in *Pisum*, TMU does not induce chromosome breaks at concentrations such as do not inhibit mitosis.

Particular attention was paid to the effect of adenine. In high concentrations (40—20 mmol/l.), this purine induces, as in *Allium*, chromosome breaks and erosion. In concentrations $\geqq 5$ mmol/l., adenine totally inhibits the function of the spindle and/or that of the centromere, and produces a pronounced chromosome contraction. Most mitoses, however, seem to be arrested at late prophase and in this respect the effect of adenine differs from that of colchicine. After treatment at concentrations just below the threshold for the c-mitotic action, lag chromatids were frequently observed. Ana- and telophase bridges were found after treatments with solutions containing 2,5 mmol/l. of adenine. These bridges seem to have arisen through a stretching of the centromeres of lagging chromatids in the direction of the spindle axis.

The cytological effects of purines have recently been studied in

tissue cultures (mouse-embryo skin, sarcoma), by BIESELE (1951). He found, *i. a.*, that 2,6-diaminopurine caused chromosome breakage in low concentrations and that adenine in higher concentrations was very effective.

Analyses of the localization of the chromosome breaks were also performed. Previously, KIHLMAN and LEVAN (1951) found that if root-tip chromosomes of *Vicia faba* were treated for 6 hours at 10° C with 0,2 % of EOC, most of the breaks induced were localized to the attachment thread of the satellite. When the treatment was prolonged, breaks in the chromosomes were also noticed at other points. A less marked localization of breaks to the satellite fibre was found after TMU treatments.

In the *Pisum* investigations, satellite fragments were found after treatments with the active purines tested, with the possible exception of adenine. Of the total number of fragments recorded, however, the percentage of satellite fragments was considerably lower than in the *Vicia* experiments, seldom exceeding 50.

In addition to the satellite fibre, breaks seemed to be localized to a region close to the centromere in the chromosomes. In order to analyse the distribution of breaks in greater detail, it was necessary to determine the morphology of the *Pisum* chromosomes. With the aid of the oxyquinoline technique of TJIO and LEVAN (1950), the seven chromosome pairs of *Pisum sativum* were identified and characterized. It appeared that the secondary constriction of one of the chromosomes differs from the secondary constrictions of others in three ways: (1) by its length, (2) by its failure to contract during treatment with oxyquinoline, and (3) by the probability of its being in contact with the nucleolus during prophase. This »secondary constriction» chromosome is called the satellite chromosome although, according to HÅKANSSON and LEVAN (1942), it has nothing to do with the formation of the nucleolus.

By means of the oxyquinoline technique, a detailed analysis was made of the distribution of breaks not lying in the satellite fibre. In this analysis, only those cells were considered in which most or all of the chromosomes could be identified, a total of 50 breaks in 40 cells being analysed. 28 of these breaks, *i. e.*, 56 %, were localized close to a centromere. As regards the distribution of the breaks on different chromosomes, it was found that, in particular, the satellite chromosome and the largest chromosome were frequently broken. Including the breaks in the satellite fibre, considerably more than the half of the EOC-induced breaks were localized to the satellite chromosome.

Finally, a comparison was made between the radiomimetic effect of EOC, nitrogen mustard and maleic hydrazide on *Pisum*. It may be mentioned that the same number of breaks (about one break per cell) as induced by EOC in a concentration of 7,5 mmol/l., was induced by maleic hydrazide in a concentration of 0,2 mmol/l. and by nitrogen mustard in a concentration of only 0,01 mmol/l. EOC, however, has the advantage over nitrogen mustard in that it has a low toxicity. From a qualitative point of view, the effect of EOC differs considerably from that of maleic hydrazide.

III. THE MODE OF ACTION OF PURINE DERIVATIVES.

1. THE ENTRANCE INTO THE NUCLEUS.

The entrance into the cell probably presents no difficulties for purine derivatives, at least for those with an almost neutral reaction. The passage into the nucleus, however, seems to be blocked rather effectively. The simplest explanation of the difference in the mode of action between purines of the TMU and EOC type described above, is that only the latter are able to penetrate the nuclear membrane, so that the concentration inside the nucleus exceeds the threshold for the radiomimetic action. Purines of the TMU type can enter the nucleus only during mitosis, when the nuclear membrane disappears. This should explain why the radiomimetic effect of these purines, expressed in per cent of abnormal cells, is dependent upon the number of mitoses during the period of treatment.

This hypothesis is supported by determinations of the relative lipoid solubilities of the purines, the results of these investigations showing that the hypothesis is consistent with the lipoid theory of OVERTON (1899, 1902). It appeared from determinations of the distribution coefficients in the systems ethyl ether/water and oleyl alcohol/water, that purines of the EOC type have a stronger affinity for the »lipoid» phase, while purines of the TMU type have a stronger affinity for the water phase of the system. Only those purines having a stronger affinity for the »lipoid» phase seem to be able to penetrate the nuclear envelope. This implies, however, that the nucleus is surrounded by a lipoid membrane. Such a membrane has been demonstrated by several workers in different organisms and by different methods. In *Allium*, the present author obtained evidence for the existence of a perinuclear lipoid layer by means of staining tests with the lipoid stain Sudan black B. Further evidence was obtained by BERG (1951), who found, by

means of his benzpyrene-caffeine method, a well-designed lipoid membrane around nuclei of onion root-tip cells.

It may seem somewhat surprising that a compound which easily penetrates the cell membrane, is practically unable to penetrate the nuclear envelope. Such strong barriers of lipoidic nature as are assumed to protect the nuclei of *Allium* root-tip cells are, however, not unknown in physiology, an interesting case having been described by NACHMANSOHN (1950).

Physostigmine and di-isopropylfluorophosphate (DFP) abolish nerve conduction. This effects is due to the inhibitory action of these drugs on acetylcholine esterase, an enzyme involved in nerve function. Prostigmine, a compound related to physostigmine, and tetraethylpyrophosphate (TEPP) are extremely potent inhibitors of acetylcholine esterase, but do not affect nerve conduction. The explanation seems to be that physostigmine and DFP, in contrast to prostigmine and TEPP, are able to penetrate into the axoplasm. The former compounds are lipoid soluble, the latter not. The structural barrier, which prevents prostigmine and TEPP from penetrating into the axoplasm and thus from coming in contact with the enzyme, is, according to NACHMANSOHN, probably the lipoid membrane, known to surround cell axons.

Since methylxanthines also are inhibitors of acetylcholine esterase, it should be of interest to know whether purines of the EOC type are able to penetrate the structural barrier surrounding axons and to affect nerve conduction.

2. BEHAVIOUR INSIDE THE NUCLEUS.

After the entrance into the nucleus, the purine derivatives, or at least those with surface activity, probably accumulate on intra-nuclear surfaces such as that of the nucleolus or the inner surface of the nuclear membrane. On these surfaces, the purines would at first exceed their threshold concentrations for chromosome-breaking ability, and those chromosome parts in contact with these surfaces would be broken first. This would explain why the breaks, induced in *Vicia faba* chromosomes by EOC, are localized to the satellite fibre, since this chromosome region is in contact with the surface of the nucleolus during interphase and prophase. Another explanation of the satellite breaks could be that the satellite fibres, and possibly secondary constrictions in general, have a greater affinity for purines or are more susceptible to their radiomimetic effects than are other parts of the chromosomes. It was hoped that the *Pisum* investigations should throw light upon the question, since, according to HÅKANSSON and LEVAN (1942), the satellite chromosomes in *Pisum sativum* have nothing to do with the formation and

localization of the nucleolus. Instead, nucleoli are formed at telophase at the centromeric regions of other chromosomes. These primary nucleoli fuse into one large nucleolus, which during interphase is attached to the centromeric regions of one or two chromosomes.

If, after EOC treatments, only a few breaks occur in the *Pisum* satellite fibre, and particularly if the breaks are localized to the centromeric region, it would seem to indicate that this localization is connected in some way or other with the nucleolus. As we have seen, most breaks were localized to the satellite fibre or to regions close to the centromeres. These results were at first rather confusing, but when later it was found that the satellites, at least during prophase, as a rule appear to lie on the surface of the nucleolus, the localization of breaks observed could be explained on the basis of the »nucleolus hypothesis». However, the results of the *Pisum* investigations are not entirely convincing, and until more evidence has been produced in support of this hypothesis, the other explanations of satellite breaks already mentioned cannot be excluded.

The »nucleolus hypothesis» accounting for the localization of breaks is based on the assumption that EOC and other purines having a similar effect, accumulate on surfaces. Also, regarding radiomimetic activity in general, a surface or boundary activity may be significant, since it is possible that the purine derivatives must be adsorbed on or come in contact with the chromosomes before breaks can be induced. For these reasons it should be of interest to obtain at least an approximative idea of the boundary activity of the purines.

As a measure of the boundary activity, a determination was made of the power of purines to replace methylene blue on the surface of charcoal (KIHLMAN, 1952). The radiomimetic purine derivatives showed a considerable boundary activity. It was found that all the purines which are »very active» inducers of chromosome breaks also have a high boundary activity and that those purines which are »inactive» or only »slightly active» in cytological tests have in general a low boundary activity. The most striking difference between the boundary activity of purines and their radiomimetic activity is that the former increases if N-bound methyl groups are substituted by groups with longer carbon chains while the latter decreases. The reason for this difference may be that a specific adsorption is involved. A specific adsorption on an intra-nuclear enzyme in competition with its substrate or co-enzyme (for instance, adenine, guanine or compounds containing these purines as constituents) could be the cause of the radiomimetic activity of

purine derivatives. It is also possible that the radiomimetic effect is favoured only by a high boundary activity, but that the chromosome-breaking ability itself is dependent upon other properties of the purine molecule, which could be altered by the lengthening of the carbon chains in N-bound alkyl groups. But if this is so, which properties could be taken into consideration?

The experiments have shown that the radiomimetic activity of purines is not dependent on any particular atomic group but on the molecule as a whole. Chemically, the purines are rather inactive and it is difficult to imagine that they should be able to break chemical bonds by a direct reaction. However, since chromosome breaks are probably breaks between molecules (see MULLER, 1941) and since the forces operating between molecules are of another character and considerably weaker than the very strong bonds between atoms, a direct action of purine derivatives cannot be excluded.

A striking correlation has been found between the radiomimetic activity of purines and their solubilizing power (WEIL-MALHERBE, 1946). Therefore, one could assume that the radiomimetic effect is a direct result of a solubilizing action of purines on the genetic material. Investigations of the solvent power of purines using, as solubilizates, the dye Congo rubin and desoxyribonucleohistone, indicates, however, that the radiomimetic effect cannot be explained in this way (KIHLMAN, 1952). Among other things, it was found that the solubilizing power — in contrast to the radiomimetic effect — does not disappear when N-bound carbon chains are lengthened, although a certain decrease could be observed. Thus, the solvent power cannot account for the difference in the radiomimetic effect found between the N-methylated purine derivatives and their corresponding higher homologues.

On the other hand, the good correlation between solvent power and radiomimetic activity of purines cannot be due to mere chance, however. According to WEIL-MALHERBE, the solvent power of purines is dependent upon the same intermolecular attraction forces which are responsible for the formation of molecular compounds. It is concluded that these »intermolecular attraction forces» also play a significant rôle in the radiomimetic activity of purines.

This does not necessarily mean that the purines act directly on intermolecular bonds existing in chromosomes. A competitive inhibition of enzymes is probably dependent upon molecular attraction between enzyme protein and inhibitor substance. Purines may also inactivate enzymes by forming molecular complexes with their prosthetic

groups or co-enzymes. According to BURTON (1951), the inhibitory action of caffeine on d-amino acid oxidase is due to the formation of a non-fluorescent complex between caffeine and the prosthetic group, in this case adenine flavine dinucleotide.

Unfortunately, very little is known about the effects of purine derivatives on intra-nuclear enzyme systems. In one experiment a test was made of the effect of EOC and TMU on the desoxyribonucleic acid phosphatase complex in nuclei (KIHLMAN, 1952). The activity of the enzyme was determined according to the histochemical method worked out by GOMORI (1946). However, these purines appeared to have no effect upon phosphatase activity.

Future investigations on the mode of action of purine derivatives on chromosomes are planned by the author.

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CHROMOSOME MORPHOLOGICAL STUDIES IN CHONDRILLA JUNCEA AND SOME REMARKS ON THE MICROSPOROGENESIS

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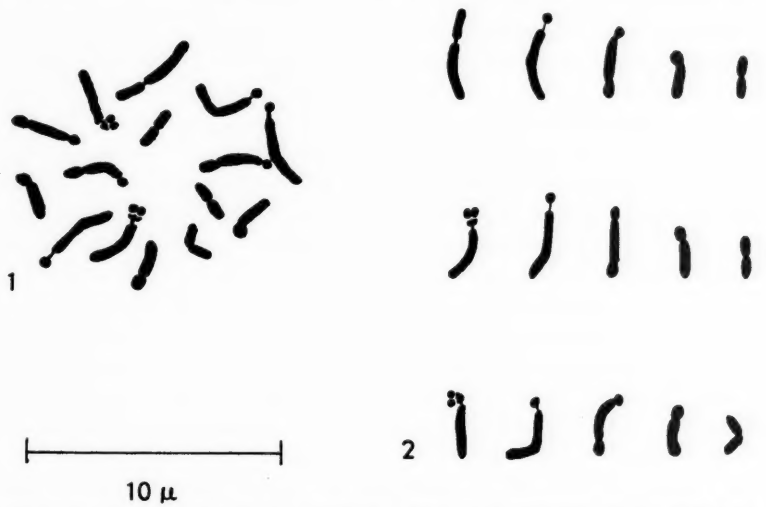
FOR a long time it has been a well-known fact that there is a close correlation between apomixis and polyploidy. According as new cases of apomixis have been discovered, this correlation has been more and more supported. A central problem in apomixis research is that of deciding whether it is a question of allo- or autopolyploidy. According to the oldest opinion, the apomicts are of hybrid origin and therefore allopolyploids. Of late years, however, autopolyploidy has with great probability been established in some cases (*cf.* GUSTAFSSON, 1947). Among the students of apomixis, opinions are now divided as to which type of polyploidy has played the most important rôle. The problem is of importance for understanding the genesis and development of apomixis. However, to decide if an apomict is auto- or allopolyploid is often no easy matter. Taxonomical and geographical studies, studies on chromosome associations in meiosis, as well as on chromosome morphology, have in certain cases yielded valuable information. Special significance should be attached to chromosome morphological studies. Up till now, however, such studies are few, a fact which may be accounted for, by the high chromosome numbers of many apomicts and perhaps the absence of morphological characteristics of the chromosomes.

From a chromosome morphological point of view, the genus *Chondrilla* with $n = 5$ as its basic number offers excellent opportunity for study. From the investigation of PODDUBNAJA-ARNOLDI (1933) it appears that satellite chromosomes occur, as well as differences in size between the chromosomes. The present author has therefore begun such a cytological investigation of *Chondrilla*, but so far has only obtained specimens of *Ch. juncea*. Lewitsky's fixative is said to be the best for bringing out the morphological characteristics of the chromosomes, and SÖRENSEN and GUDJÓNSON (1946) have obtained excellent results with it in the case of *Taraxacum*. However, as the fixative proved unsuitable for *Ch. juncea* I have used Navashin instead. The slides were

stained with crystal violet after hydrolysis in N.HCl at about 20° for 3 hours or more. Since the hydrolysis left the plasma quite colourless and the chromosomes relatively weakly stained, the morphological characteristics of the latter were quite apparent, as may be seen in Fig. 1. This figure illustrates a root tip metaphase and it is easy to identify the homologous chromosomes. Quite soon one also observes



Figs. 1—2. *Chondrilla juncea.* — 1, root tip mitosis. — 2, the chromosomes from Fig. 1 arranged in three rows so that the homologous ones are placed opposite each other.

that there are three homologues of every type. In order to make this more evident I have in Fig. 2 arranged the chromosomes from Fig. 1 in three rows, so that the homologous chromosomes are placed opposite each other.

From a chromosome morphological point of view, three similar genomes each with five chromosomes are present in *Ch. juncea.* As to the size, one can in every genome distinguish two classes, one with three long chromosomes, one with two short ones. Of the long chromosomes, the longest has a submedian constriction; that of medium length has a long subterminal constriction which separates a satellite, while the third has a subterminal constriction at each end. Of the two short chromosomes, one has a subterminal constriction, the other a median. One of the two short chromosomes, *viz.*, that with the median constriction, is somewhat shorter than the other.

Probably *Ch. juncea* has originated from some sexual species of the genus. Up till now, however, only one such species has been discovered, viz., *Ch. ambigua* with $2n = 10$ while nine others have been shown to be apomicts with $2n = 15$ or 20 (PODDUBNAJA-ARNOLDI, 1933). Of the sexual *Ch. ambigua*, PODDUBNAJA-ARNOLDI has drawn a pollen grain mitosis and a root tip mitosis. Judging from the five chromosomes of the pollen grain mitosis (Abb. 4), it is quite evident that they may be divided, as in *Ch. juncea*, into two size classes, one with three long chromosomes and one with two short ones. From the root tip mitosis (Abb. 1) it also appears that, as in *Ch. juncea*, one of the long chromosomes has a well separated satellite. I mention this only as an interesting observation, not as a suggestion that *Ch. juncea* is an autotriploid of *Ch. ambigua*. To decide this question, further cytological as well as taxonomical and geographical studies are required. At present, one can only say that *Ch. juncea* from a chromosome morphological point of view must be regarded as an autotriploid.

Lastly, some remarks on the microsporogenesis in *Ch. juncea* may be made. In a previous paper (BERGMAN, 1950), I proved that *Ch. juncea* occurs in two different types with reference to its microsporogenesis. One type (from Hortus Bergianus in Stockholm) formed practically only dyads, the other (from Hortus Botanicus in Uppsala) formed practically only tetrads. I interpreted the difference between the two types as being genetically conditioned and consequently considered that they belonged to two different clones. But since they grew in two geographically different localities I could not altogether deny the possibility of environmental influences. Last summer (1950), however, I found that *Ch. juncea* was also cultivated at another place in Hortus Bergianus. The dyad plants were controlled and revealed no changes; the other plants, on the contrary, formed practically only tetrads. As the environment was the same, the difference must be considered to be genetically conditioned.

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HERITABILITY OF THE UDDER PROPORTIONS IN DAIRY COWS

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INTRODUCTION.

THE ideal udder of the dairy cow is symmetrically developed and well attached to the body. It is rather common, however, that the front quarters are much smaller than the rear quarters, making the floor of the udder tilted to the rear. When cows with such udders are milked by machine, the milk flow ceases earlier from the front than from the rear teats, and the former will, therefore, be subjected to vacuum for a longer time than the latter after the cessation of the flow. It is pointed out by PETERSEN (1944) that the continued action of vacuum on the empty teats may damage the delicate lining of the teat cistern, and that such damage is an important cause of mastitis. MEIGS *et al.* (1949) have studied the effect of »severe machine milking», where the machines worked 20 minutes on each cow, with a vacuum of 40 cm Hg, and they found that on this treatment almost every cow developed mastitis; 65 per cent of the severe infections in 25 cows were localized to the front quarters and 35 per cent to the rear quarters of the udder. However, most other investigators have found a slightly higher frequency of mastitis in the rear than in the front quarters. BRATLIE (1950), *e. g.*, studied 16648 cows of which 4264 had one or more quarters infected; each one of the two front quarters was infected in 23 per cent of the cases, the left rear quarter in 26 per cent and the right rear quarter in 28 per cent. There may be other factors than »severe machine milking» which favour infections in the rear quarters, *e. g.* greater exposure to injuries from the exterior, lower efficiency of the teat sphincter, *etc.* However this may be, all investigators agree that it is important for the health of the udder that the milking machine is not allowed to run idle on the teats. Furthermore, the tilted udder is often poorly attached to the body and therefore pendulous. A pronounced pendulous udder is difficult to milk, both by hand and by machine, and it is easily injured, *e. g.* by treading in the barn or by barbed wires in the pasture. The shape of the udder is, therefore, an

economically important character which should be considered in the improvement of the breeds.

The yield of each half or quarter of the udder, in per cent of the total yield, would seem to give the best indication of the symmetry in udder development. The old method of scoring is rather inaccurate, and to measure the size of the glands is very difficult. Numerous investigations have been made on the relative yield of the quarters; the most thorough are probably those by TURNER (1934), and by MATTHEWS, SWETT and GRAVES (1941). TURNER studied 45 cows of the Holstein, Jersey and Ayrshire breeds, and he found that, on an average, 42 per cent of the milk was produced by the front quarters and 58 per cent by the rear quarters, whereas the yield of the left and the right halves of the udder was practically the same. The investigations by MATTHEWS *et al.* comprised 61 Holstein and 33 Jersey cows. There was little difference between the breeds in udder proportions. On an average, the cows produced 42,8 per cent of the milk by the front and 57,2 per cent by the rear quarters; the two left quarters produced 49,4 per cent and the two right quarters 50,6 per cent. MATTHEWS *et al.* presented also an extensive survey of the literature which shows that their results are in good agreement with previous findings in regard to the relative yield of the quarters. However, the genetic basis of the variations in asymmetry of the udder has not been analysed in any of these investigations. Students of animal genetics seem to have been more interested in rare defects of udder and teats than in the normal variations in the relative yield of the quarters. There are, however, several observations recorded in the literature which indicate that these variations also are highly hereditary.

PLUMB (1896) measured the milk yield of each quarter of 65 cows and observed that the relative yield of the front and the rear quarters usually was very similar for daughters and their dams. IVANOVA (1931), in a similar study, expressed the difference between the yield of the front and the rear quarters in per cent of the total yield, and used this percentage as a »symmetry index» for the udder. From pedigree analyses she concluded that asymmetry of udder development was hereditary and based upon a single dominant gene.

It has been pointed out by KRONACHER (1932) and by BONNIER and HANSSON (1948) that the shape of the udder is a valuable character for the diagnosis of monozygocity of cattle twins. SCHMIDT and KLIESCH (1938) state: »Nach den bisher gemachten Beobachtungen ist die Ausbildung des Euters bei eineiigen weiblichen Zwillingstieren auffallend ähnlich. Das gilt nicht nur für Ausformung des gesamten Euters, son-

dern auch för die Ausbildung der einzelnen Striche und Viertel». HANCOCK (1949) has mentioned two sets of monozygotic cow twins in which one member of each pair lacked one of the quarters whereas the other member of the pair was normal. The animals had been under observation from three months of age. In another case, however, both members of a monozygotic pair had non-functional front quarters which never had produced any milk. HEIZER (1932) found in a Guernsey herd several cows with one quarter entirely missing, and with the two teats on the opposite side placed very close together. One cow with defective udder produced two defective and two normal daughters, and a son of the same cow produced in the same herd several daughters with defective udders. The defect was supposed to be due to a recessive gene in homozygous condition. TUFF (1950) has reported that two bulls, sire and son, each produced several daughters with missing front quarters, and another case where a bull apparently was heterozygous for a dominant gene for supernumerary teats, placed between the normal front and rear teats and sometimes more or less fused with one of them. Fused teats have been found also in beef cattle. JOHNSON (1945) reported that in the South Dakota station herd five Hereford cows had fused teats on one side. The two teats on the opposite side were in all five cases placed very close together. The cows with fused teats were all closely related to one another. Their dams had normal teats, although placed unusually close together. JOHNSON suggests that the teat fusion was due to homozygocity for a recessive gene, which in single dose caused the teats on both sides to be placed very close together.

LEIGHTON and GRAVES (1947) studied the udder development by a different method. All animals in the dairy herd at the Agricultural Research Center, Beltsville, U. S. A., were photographed at certain intervals according to a standard procedure, and measurements were taken, on the photographs, of the slope of the rump from the dorsal extremity of Tuber coxae to the corresponding point of Tuber ischii, and of the slope of the floor of the udder from the base of the front teats to the base of the rear teats. No correlation was found between the slope of the rump and the slope of the udder. Based on comparisons between daughters and dams, and between full sisters, these authors conclude that the slope of the udder, although it may be modified by mastitis and other causes, is a highly hereditary character which probably depends on many genes.

This brief literature review would seem to indicate that the normal variations in the shape of the udder, as well as such abnormalities as

entirely missing or non-functional quarters, unusual development of supernumerary glands (polymastia), and fused teats, are hereditary. — The object of the present investigation was to study the normal variation in the relative yield of the four glands of the udder within a fairly large sample of cows from each one of the three Swedish dairy breeds, in order to show to what extent this variation is hereditary.

MATERIAL AND METHODS.

Our investigations on udder evacuation started in 1947 in the station herd of the Agricultural College. A special milking machine was constructed which made it possible to measure the rate of milk flow from each quarter of the udder, and also to take continuous samples of the milk for chemical analysis (JOHANSSON, KORKMAN and NELSON, 1952). For the present study it was desirable to obtain data for a fairly large number of cows from each breed. The experimental milkings could be simplified, however, because it was not necessary to determine the rate of flow, but only the total amount of milk from each quarter. We are indebted to A. B. Manus, Norrköping, for designing and manufacturing the milking machine shown in Fig. 1, on the following pages called »the field machine», which proved to be well fitted for the purpose. This machine was used for experimental milkings in 5 herds of Swedish Red and White Cattle (SRB), 8 herds of Swedish Friesians (SLB), and 9 herds of Swedish Polled Cattle (SKB).

The herds were picked in order to obtain fair samples of the breeds. Herds on different planes of improvement were represented in each group; in some of the herds all animals were registered in the herd book or eligible for registration, and in other herds the cows may best be described as »high grades». The sires, however, were always registered. In each herd all cows, which could be used for our purpose, were tested. For reasons which will be discussed later, no test milkings were made on SRB or SLB cows with lower yield than 8 kg milk per day or on SKB cows with less than 6 kg. Cows with non-functional quarters or where the yield of one or several quarters was known to have been decreased by mastitis, were also excluded from the test, *i. e.* an attempt was made to avoid all cows with secretory disturbances due to diseases in any one of the quarters. Furthermore, only groups of paternal half sisters, each group comprising at least three individuals, and dam-daughter pairs were tested. — In addition, test milkings were made also on 12 sets of, according to the diagnosis, monozygous cow twins and

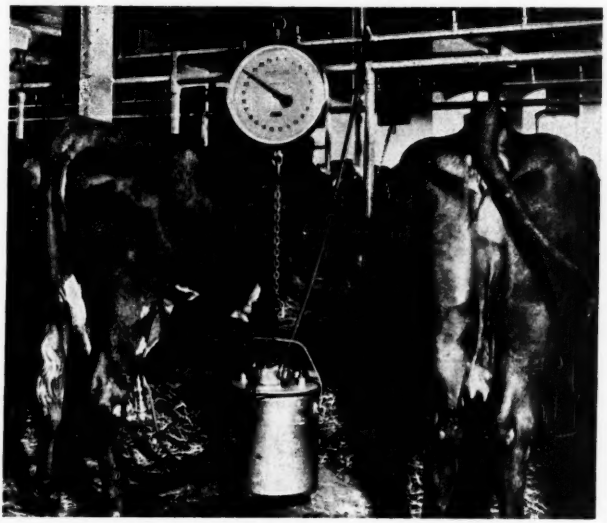

Fig. 1. »The field machine» at work. The milk bucket is cylindrical and contains four removeable steel insertions, or sectors, one for the milk of each quarter of the udder. The bucket is hanging in a spring balance, thus making possible a determination of the rate of milk flow from the whole udder during milking.

one set of identical triplets at Wiad. Owing to disturbances from mastitis, however, the data from 5 sets of the twins could be used only partly in the analysis of the results.

Each cow was milked with »the field machine» at two, or in some cases three milkings on consecutive days. Table 1 shows the number of cows tested from each breed, and the number of herds from which the cows were drawn. On the basis of the actual yield of each quarter the following percentages, or indices were calculated for each cow:

(1) The yield of the two front quarters in per cent of the total yield of the udder (front-to-rear index),

(2) The yield of the two left quarters in per cent of the total yield (left-to-right index),

(3) The yield of the left front quarter in per cent of the yield of the front half of the udder (front index),

(4) The yield of the left rear quarter in per cent of the yield of the rear half (rear index).

The first percentage is an index of the symmetry between the front

and the rear parts of the udder, the second shows the symmetry between the left and the right halves, and the third and fourth percentages express the symmetry between the left and right quarters within the front and rear half, respectively.

These data on the relative yield of the halves and quarters of the udder have been analysed for differences between groups and subgroups in regard to averages and variances, correlation between variables, *etc.* The partioning of variance on various sources is made according to WINSOR and CLARKE (1940), and for analysis of variance-heterogeneity and logarithmic transformations we have used the methods developed by BARTLETT (1937) and BARTLETT and KENDALL (1946).

The shape of the udder, measured by the relative yield of the halves and quarters, is treated as a quantitative character. Owing to the nature of our data we have not attempted a strictly Mendelian analysis but rather to measure the part of the variance which is determined by heredity, *i. e.* to estimate the heritability. For such estimates we have used the correlations between parental half sisters, and between dams and daughters (*cf.* LUSH, 1949). FISHER (1951) has pointed out that the »coefficient of heritability» is »one of those unfortunate short-cuts, which have often emerged in biometry for lack of a more thorough analysis of the data». A warning against too mechanical calculation and use of this coefficient is probably needed, but when handled cautiously we think that the heritability coefficient is very useful for many purposes, just as other ratios may be, *e. g* the coefficient of correlation. At any rate, the reader can hardly run the risk of being misled by our heritability estimates, because we give full particulars about the components of variance on which they are based.

VARIATIONS IN THE RELATIVE YIELD OF THE QUARTERS.

The frequency distribution of the percentages indicating the relative yield of the halves and quarters of the udder, are shown in Figs. 2 and 3, and the averages and standard deviations for each breed are presented in Table 1. For comparison Table 1 presents also the corresponding averages for 7 sets of monozygous twins and one set of triplets, all of the SRB breed, except one set of SLB twins. The discussion of these sets, however, will be postponed to a following paragraph.

There is a pronounced difference between the symmetry indices of

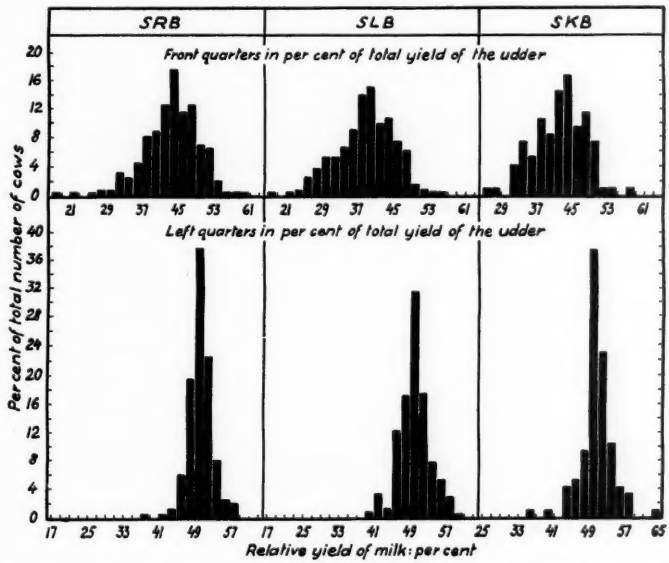
SRB
SLB
SKB
Front quarters in per cent of total yield of the udder
Left quarters in per cent of total yield of the udder
Per cent of total number of cows
Relative yield of milk: per cent

Fig. 2. Frequency distribution of the front-to-rear and the left-to-right indices, within each breed.

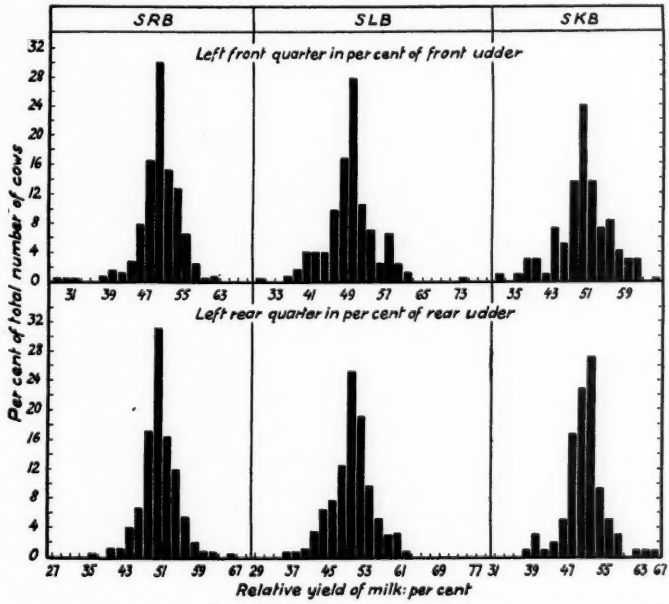
SRB
SLB
SKB
Left front quarter in per cent of front udder
Left rear quarter in per cent of rear udder
Per cent of total number of cows
Relative yield of milk: per cent

Fig. 3. Frequency distribution of the front and rear indices, within each breed.

TABLE 1. *Relative yield of the halves and quarters of the udder within the Swedish dairy breeds; averages* ($\bar{y}$) *and standard deviations* (s).

Breed	Number of		Per cent of total yield of the udder obtained from				Left front quarter in per cent of front half		Left rear quarter in per cent of rear half	
			Front half		Left half					
	herds	cows	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s
SRB	5	250	43,3	6,14	50,0	2,69	49,9	4,38	50,1	3,74
SLB	8	245	38,7	6,44	49,7	3,42	49,3	5,33	50,0	4,60
SKB	9	96	41,9	5,80	50,3	3,56	49,9	5,93	50,5	4,40
Total and av.	22	591	41,2	6,19 [1]	49,9	3,16 [1]	49,6	5,04 [1]	50,1	4,17 [1]
7 sets of monozygous twins and one set of triplets (SRB)		17	43,0	4,03	52,4	4,10	52,4	5,64	52,6	4,88

[1] The standard deviation is calculated »within breeds», *i. e.* the breed differences are eliminated.

the front-to-rear and the left-to-right halves of the udder. The index (1) for the front-to-rear halves is, on an average for the three breds, 41,2, and the differences between breeds are significant (*cf.* Table 5), whereas the corresponding index (2) for the left-to-right halves is 49,9, with insignificant breed differences. The standard deviation of the former index reaches about twice the value of that of the latter. The relative yield of the two quarters within the front and rear part of the udder, respectively, varies (*cf.* Fig. 3) but, on an average, the capacity of yield of the two quarters is the same. The deviations of the indices 3 and 4 from their most probable value, *i. e.* 50 per cent, seem to be more or less at random. However, a front quarter produces, as a rule, lower yield than a rear quarter. These facts seem to indicate that there is a fundamental difference between the causes of variation in the front-to-rear and the left-to-right indices.

CROSSMAN, DODD, LEE and NEAVE (1950) state that, when the quarters are not damaged by mastitis, there is usually no great difference in yield between the two front quarters, or the two rear quarters. The same authors have shown, however, that the relative yield of a certain quarter may decrease very markedly during the course of a lactation due to bacterial infections, even when no clinical symptoms of mastitis are

TABLE 2. *Average* ($\bar{y}$) *and standard deviation* (*within breeds*) *of the symmetry indices in different lactations. Total data for the three breeds; two milkings per cow.*

Lactation (x)	Number of cows	Front-to-rear		Left-to-right		Front index		Rear index	
		$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s
1st	168	42,0	5,65	49,8	2,54	49,6	5,15	50,0	3,14
2nd	161	41,1	6,25	50,3	3,46	49,9	4,85	50,6	4,74
3rd	85	41,7	6,21	49,7	3,21	50,0	4,89	49,6	4,40
4th	49	40,3	6,37	49,5	3,42	48,9	4,97	50,0	4,48
5th	35	39,7	6,43	49,8	3,02	49,8	4,05	49,0	4,36
6th—8th	93	40,3	6,89	49,9	3,96	49,4	6,36	50,2	5,01
Regression (within breeds): $b_{\bar{y}/x}$ and $b_{s/x}$		-0,37***	0,25*	-0,06	0,19*	0,07	0,10	0,05	0,29**
BARTLETT's test for variance-heterogeneity: χ^2		—	10,22	—	43,99***	—	29,08***	—	70,40***

* = $P < 0{,}05$, ** = $P < 0{,}01$, *** = $P < 0{,}001$.

manifested. MATTHEWS, SWETT and GRAVES (1941) studied on 24 cows the correlation between the relative yield of an individual quarter at different times, and found that for consecutive days, or morning and evening milkings, the correlation was very high ($r=0{,}98$), for milkings three months apart a little lower ($r=0{,}90$), and for milkings in different lactations, 3 months after calving, rather low ($r=0{,}40$). In our material from the station herd 33 cows had been tested one or several times at corresponding stages of two consecutive lactations for the relative yield of the quarters when the total yield per milking was at least 2 kg milk. The coefficient for the correlation between the indices for the two lactations was for the front-to-rear index 0,769 ($P < 0{,}001$) and for the left-to-right index 0,188 ($P > 0{,}05$). These coefficients show that the front-to-rear udder index is much less variable from one lactation to another than is the left-to-right index.

If mastitis is an important source of variance in the symmetry indices, the variance would be supposed to increase with increasing ordinal number of the lactation. Table 2 presents an analysis of this particular question. The regression (within breeds) of the average indices, and of the standard deviations, on the ordinal number of the lacta-

tion was calculated for each index. The front-to-rear index shows a slight but significant decline with increasing age of the cows, but the other three indices do not show any such trend. However, not only the standard deviation of the front-to-rear index but also that of the left-to-right, and the rear index increase significantly with age. As a check on this result, BARTLETT's test for the heterogeneity of variances was applied to the same data. The heterogeneity is highly significant for the indices of symmetry between the left and the right side of the udder but not for the front-to-rear index. There are, apparently, sampling differences between the six groups of cows, but in spite thereof the trend to increasing variance with increasing number of lactations is clearly manifested. It seems likely that this increase is caused by clinical or sub-clinical mastitis.

Another question is whether the relative yield of the front half, or any individual quarter of the udder, tends to increase or decrease in the course of the lactation period. TURNER (1934) studied the yield of the individual quarters throughout the lactation period for 18 Holstein and 20 Jersey cows, and calculated the average percentage yield of each quarter for each month of lactation. None of the four averages showed any trend to increase or decrease during the lactation. TURNER suggests, however, that the relative amount of secretory tissue in each quarter is most accurately expressed by the yield of the quarters at the stage of maximum production in the lactation period. This seems to be verified by Tables 3 and 4 where it is shown that the variance increases with progressing lactation. CROSSMAN *et al.* (1950) state that they could notice a tendency for the front quarters to dry off a little faster than the rear quarters towards the end of the lactation, but no figures were published to show this tendency. In our own material from the station herd we have data on the relative yield of the quarters for 15 cows throughout the first 10 months of a lactation period. The averages and standard deviations for the milk yield, and the symmetry indices of the udder at different stages of the lactation are shown in Table 3. Table 4 presents corresponding figures for the field data.

In Table 3 there is a slightly significant trend of the front-to-rear index to decrease towards the end of the lactation, and a clearly significant similar trend of the left-to-right index caused mainly by a comparatively rapid decrease in the yield of the left rear quarter. Table 4, where the lactation stages are represented by different cows, shows the same trend in the front-to-rear index during the progress of lactation, but otherwise the decreasing trend is significant only for the rear

TABLE 3. *Relative yield of the quarters at different stages of the lactation. Averages ($\bar{y}$) and standard deviations (s) for 15 cows.*

Stage of lactation: days from last calving	Total yield of the udder per milking, kgs		Relative yield of the halves and quarters of the udder (y)							
			Front half in per cent of total: Front-to-rear index		Left half in per cent of total: Left-to-right index		Left front quarter in per cent of front half: Front index		Left rear quarter in per cent of rear half: Rear index	
x	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s
1—60	9,22	2,05	44,9	4,91	50,3	2,27	49,3	3,47	51,0	3,14
61—120	8,85	1,83	45,1	5,70	49,1	2,80	48,8	3,97	49,1	5,75
121—180	7,57	1,57	44,9	6,36	49,1	2,51	48,7	4,09	49,3	4,92
181—240	5,14	1,80	43,8	8,67	48,1	2,53	48,5	4,69	48,1	4,98
241—300	3,01	1,72	42,6	13,33	47,6	4,06	49,9	5,64	46,8	7,98
Regression: $b_{\bar{y}/\bar{x}}$ and $b_{s/\bar{x}}$			-0,59*	1,98*	-0,64**	0,33	0,09	0,51**	-0,94**	0,71
BARTLETT's test for the variance-heterogeneity: χ^2			—	18,77***	—	6,38	—	3,83	—	8,65

* = P < 0,05, ** = P < 0,01, *** = P < 0,001.

TABLE 4. *Relative yield of the quarters at different stages of the lactation. Averages ($\bar{y}$) and standard deviation (s) for the total number of cows classified according to the stage of lactation. The variances are calculated »within breeds».*

Stage of lactation: days from last calving	Number of cows	Total yield of the udder per milking, kgs		Relative yield of the halves and quarters of the udder (y)							
				Front half in per cent of total		Left half in per cent of total		Left front quarter in per cent of front half		Left rear quarter in per cent of rear half	
x		$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s	$\bar{y}$	s
1— 60	177	10,13	2,24	42,4	4,81	50,0	2,62	49,6	4,29	50,4	3,45
61—120	150	9,46	2,08	41,2	6,24	50,1	2,91	50,0	5,53	50,3	4,14
121—180	150	8,16	1,89	40,3	6,10	49,9	3,17	49,6	4,85	50,1	4,47
181—240	77	7,35	1,88	41,6	6,80	49,3	3,89	48,6	5,32	49,8	5,26
241—300	24	5,73	1,74	37,6	7,74	50,1	3,59	50,4	5,57	49,5	3,96
Regression (within breeds):[1] $b_{\bar{y}/\bar{x}}$ and $b_{s/\bar{x}}$				-0,68*	0,74**	-0,12	0,34***	0,01	0,31	-0,21*	0,41**
BARTLETT's test for variance-heterogeneity: χ^2				—	20,82***	—	19,40***	—	11,92*	—	21,83***

[1] $\bar{x}$ = class average.

* = P < 0,05, ** = P < 0,01, *** = P < 0,001.

index. The standard deviation increases during the progress of lactation in regard to all indices, but in Table 3 the increase is not significant for the left-to-rear or the rear index and in Table 4 not for the front index. The heterogeneity of the variances is significant for all four indices in Table 4 but only for the front-to-rear index in Table 3.

That the standard deviation of the indices increases from the beginning to the end of the lactation is probably due partly to an increasing variation in the relative amount of residual milk with decreasing total yield, and partly to the effect of sub-clinical mastitis. Chance variation between the glands of the same udder in the rate of drying off towards the end of the lactation may be another factor. Because of this increase in variation of the indices towards the end of the lactation, tests of the relative yield of the quarters should preferably be made within 6 months from calving. — That the front quarters may have a slight tendency to dry off a little faster than the rear quarters is, however, of little importance.

A priori it seems possible that a sub-normal development of, *e. g.*, the left front quarter may have a compensatory effect on the development of the left rear quarter. Therefore, the co-variance between the symmetry index (3) of the two front quarters and the corresponding index (4) of the two rear quarters was analysed. However, no significant correlation coefficients were obtained, the correlation »within breeds» being only — 0,03. The result conforms to the hypothesis that asymmetry between the two front quarters is independent of an asymmetry between the two rear quarters.

This survey of the variations in the relative yield of the quarters has shown that it is influenced by several non-genetic factors. The most important of these factors are, apparently, the udder infections. As stated before, we have tried to avoid, as far as possible, to include in our material cows with one or several quarters damaged by clinically manifested mastitis, but it is very likely that some cases have escaped attention. At any rate, we have had no control of cases of sub-clinical mastitis, which may change the relative yield of a quarter quite considerably. Several investigations indicate that there are genetic differences between cows in the degree of their susceptibility to mastitis (*cf.* LUSH, 1950), but there are no reasons to assume that, in a certain cow, one quarter is more susceptible than another. Most types of bacterial infections seem to have about the same frequency on all the four quarters. Owing to the random distribution on the quarters, the infections have only a moderate influence on the front-to-rear and the left-to-right

indices, but the influence on the indices for individual quarters is great. This is one reason why we have, contrary to previous investigators, based our analyses mainly on the relative yield of the halves of the udder, and not on that of the individual quarters.

THE CORRELATION BETWEEN RELATIVES.

An analysis of the genetic causes of variation in the relative yield of the halves and quarters of the udder has to be based on the similarities between relatives. The test animals were chosen to represent two degrees of consanguinity, *viz.* paternal half sister and dam-daughter pairs. In a random bred population the genetic correlation between half sibs is 0,25 and between dams and daughters 0,5. The herds from which our material is drawn are, of course, not strictly random bred, because planned selection has been carried out, but the degree of inbreeding is very low, and therefore the actual coefficients of relationship may be supposed to deviate only slightly from those stated above. The genetic correlation between monozygous twins, or triplets, is 1. Certain errors, however, may be involved in the diagnosis of monozygosity.

Table 5 presents an analysis of the variance in the udder indices of paternal half sibs. In regard to the front-to-rear index there are highly significant differences between breeds, between groups of paternal half sibs (within breeds), and between paternal half sibs. For the other three indices there are significant differences only between cows. The variance within cows is rather small, especially for the front-to-rear index, but this is partly due to the fact that only two test milkings were made for each cow, on consecutive days. It was shown in the previous paragraph that the repeatability of the front-to-rear index, determined in different lactations, was 0,769, and for the left-to-right index only 0,188. These coefficients indicate that at least one fourth of the variance in the front-to-rear index, and the major part of the variance in the left-to-right index is due to non-genetic causes. The variance between groups of paternal half sibs corresponds roughly to a fourth of the additive genetic variance within the population; it includes a small portion of the effects of gene interactions, if any, but nothing of dominance deviations (*cf.* LUSH, 1949). Consequently, on the basis of the correlation between half sibs (0,221), the heritability of the front-to-rear index of the udder may be estimated to 0,88. However, the correlation coefficient has a considerable sampling error, and in the estimate this error is multiplied by four. Therefore, this »coefficient of heritability» must be inter-

TABLE 5. *Analysis of variance in the udder indices of paternal half sisters.*

Source of variance	Degrees of freedom	Mean squares				Percentage distribution of the variance within breeds			
		Front-to-rear index	Left-to-right index	Front index	Rear index	Front-to-rear index	Left-to-right index	Front index	Rear index
Total variance	1181	43,5	10,9	27,2	18,7	—	—	—	—
Between breeds	2	2693,5***	30,5	54,5	18,5	—	—	—	—
Between sires	59	226,0***	22,0	64,4	31,0	22,1	1,2	3,0	—
Between paternal half sisters	529	59,9***	19,5***	49,4***	35,3***	73,5	80,2	84,2	85,7
Within cows	591	1,8	2,0	3,5	2,7	4,4	18,6	12,8	14,3

*** = $P < 0{,}001$.

preted with caution, and can probably not be accepted on its face value. It shows, however, that the relative yield of the front half of the udder is highly hereditary.

The other three indices tend, within the limits of random error, towards the same average value (50 per cent) for all the groups of half sibs, indicating that the variation in these indices is not hereditary. This result is somewhat surprising, and it might be suspected that it does not reveal the whole truth. If the cows in some groups had inherited from their sires a tendency to under-development of one quarter, left or right, front or rear, but if it depended entirely on chance which gland was affected, the genetic differences between sires would not be revealed by the analysis in Table 5. Such genetic variation, however, would be expected to manifest itself in significant differences between the variances of the groups. Therefore, the heterogeneity of the variances of the progeny groups of 51 sires, containing at last 4 half sibs, were tested in regard to the front and rear indices, according to BARTLETT (1937), and it was found to be highly significant. The χ^2 value ($f=50$) was for the front index 115,1*** and for the rear index 101,0***. In this test the half-sib groups of the three breeds were treated together.

The next step was to apply the analysis of variance to the transformed variances, according to BARTLETT and KENDALL (1946). All half-sib groups containing at least 5 cows were used for this purpose. Groups with at least 10 cows were divided at random into 2 sub-groups

TABLE 6. *Analysis of variance of the transformed variances* ($log_e\ s^2$) *of the symmetry indices of the front and rear udder.*

Source of variance	Degrees of freedom	Mean square	Percentage distribution of the variance
Total variance within front and rear udder	188	1,04	100,0
Between breeds	4	3,54*	7,1
Between sires	92	0,95	—
Within sires (between groups of 5 daugthers)	92	1,03	92,9

* = $P < 0{,}05$.

with 5 cows each, groups with at least 15 cows into 3 sub-groups, *etc.*; no more than 5 cows were allotted to any group or sub-group. The analysis was based on the Napierian logarithm (log_e) for the variance (s^2) of each sub-group. The analysis of the transformed variances was made for the front index and for the rear index separately. No significant differences between sires were found in either case, but for the front index the differences between breeds were significant ($P < 0{,}01$). The pooled result of these two analyses is shown in Table 6. There are no differences between sires in the variances of the two indices for the relative yield of the front, or the rear quarters, but there is a significant difference between breeds. — The significant heterogeneity of the variance of half-sib groups, shown by BARTLETT's test when all breeds were

TABLE 7. *Correlation between dams* (x) *and daughters* (y) *in the front-to-rear and the left-to-right indices.*

Source of variance	Degrees of freedom	Correlation and regression			
		Front-to-rear index		Left-to-right index	
		r_{xy}	$b_{y/x}$	r_{xy}	$b_{y/x}$
Total	69	0,350**	0,245	— 0,070	— 0,066
Between breeds	1	0,850	0,531	0,967	0,535
Within breeds	67	0,242*	0,173	— 0,163	— 0,161
Between sires	27	0,098	0,070	— 0,302	— 0,239
Within groups of daughters of the same sire	39	0,414**	0,299	— 0,063	— 0,074

* = $P < 0{,}05$, ** = $P < 0{,}01$.

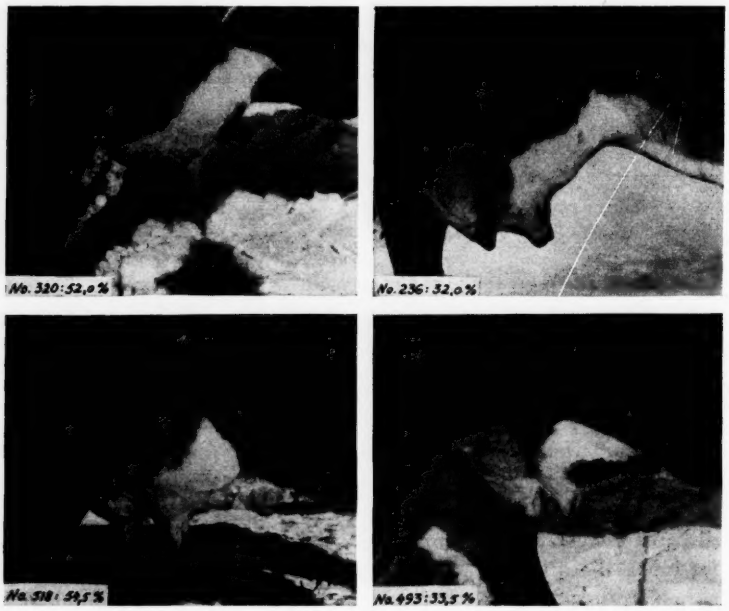

Fig. 4. Example of dam daughter pairs with striking similarity in the shape of udder. *To the left*: above the dam No. 320 with the front-to-rear index 52,0, and below her daughter (No. 518) with the index 54,5. *To the right*: above the dam No. 236 with the front-to-rear index 32,0, and below her daughter with the index 33,5. In both dam-daughter pairs the teats are also very similar in shape. The cows 320 and 518 have long front teats and short rear teats, whereas in the other pair, cows 236 and 493, all four teats are rather short, and the milk cisterns of the udder are distended at the base due to thin, flabby walls.

treated together, seems to be caused mainly by breed differences, and to some extent also by age differences between the groups (*cf.* Table 2).

The conclusion is that the variation in the relative yield of the two front quarters, and the two rear quarters, is not hereditary to any significant extent. The variation is probably due partly to coincidences in the development of the glands, and partly to udder infections which affect all four glands with about equal frequency.

The material contained 71 pairs of dams and daughters, and the correlation analysis for this degree of relationship is shown in Table 7. The dam-daughter correlation »within sires» may be supposed to provide the best indication of the genetic fraction of the variance. For the

TABLE 8. *Analysis of variance in the udder indices of monozygous twins and triplets.*

Source of variance	Front-to-rear index [1]			Pooled variance for front and rear indices [2]		
	Degrees of freedom	Mean square	Per cent of total variance	Degrees of freedom	Mean square	Per cent of total variance
Total variance	53	27,3	100,0	66	28,3	100,0
Between sets	12	76,2	33,9	14	65,4	13,6
Between members of a set.....	14	36,5***	63,3	18	48,3***	78,2
Within cows	27	0,8	2,8	34	2,4	8,2

[1] 12 sets of twins and one set of triplets. For 5 sets of twins the index is calculated only for one side of the udder (left or right) due to mastitis in one or both quarters on the other side.

[2] 7 sets of twins (5 sets discarded owing to mastitis) and 1 set of triplets.

*** = $P < 0{,}001$.

front-to-rear index this correlation is clearly significant ($P < 0{,}01$). The doubling of the coefficient yields 0,83 is an estimate of the heritability of the character, which agrees very well with the estimate (0,88) based on the correlation between half-sibs. For the left-to-right index there is no significant correlation between dams and daughters, and this is also in perfect agreement with the results from the half-sib analysis.

The analysis of the data for 12 pairs of supposedly monozygous twins and one set of triplets is presented in Table 8. The agreement with the results from the analysis of the field data is not very good, because significant differences are found only between members of a homozygous set but not between the sets, whereas the reverse would be expected. The data are peculiar in showing the same standard deviation for the front-to-rear as for the left-to-right index (*cf.* Table 1) even since 5 sets of twins with one or more quarters damaged by mastitis were eliminated. The variance between sets amounts to 33,9 per cent of the total variance in the front-to-rear index and to 13,6 per cent in the front and rear indices. These figures would express the total genetic variance in the population studied, including dominance deviations and the effect of gene interactions. Since there are no significant differences between the monozygous sets, it would be too hazardous to estimate the heritability on the basis of the intra-set correlation. The udders of the

twins and triplets were, apparently, more damaged by infections than the udders of the half-sibs and the dam-daughter pairs. This is probably the main explanation of the comparatively great variation within the sets.

DISCUSSION.

Nature's »aim» is, apparently, to make the left and the right side of the udder equal in development and capacity of yield. The left quarters of the udder produce, on an average, approximately 50 per cent of the total amount of milk, but there is a considerable variation around this average ($s=3{,}16$). The major part of the variation is apparently caused by clinical or sub-clinical attacks of mastitis, but part of it is probably due to coincidences during the development of the glands. The results obtained in the analysis of the data point very consistently to the conclusion that the variation in the relative yield of the left and the right halves of the udder is wholly non-genetic. The repeatability of the left-to-right index from one lactation to the other was insignificant, as well as all correlations between relatives. There was a significant variance-heterogeneity between the half-sib groups in regard to the relative yield of the two front quarters, as well as that of the two rear quarters, indicating that some bulls may have passed genes to their offspring for under-development of any quarter of the udder, although not for under-development of a certain quarter on the left or the right side. Further analysis showed, however, that the heterogeneity was due to differences between the breeds and the age groups of cows, and not to genetic differences between the sires.

The front half of the udder yields, on an average for the Swedish dairy breeds, about 41 per cent of the total milk, and the rear half consequently about 59 per cent. The variation in this »symmetry index» is, however, much greater than in the left-to-right index (*cf.* Fig. 2); the standard deviation within breeds is 6,19. The correlations between half-sibs and between dams and daughters indicate that 83 to 88 per cent of the total variance is additively genetic, but two other results may tend to show that this estimate may be a little too high. The repeatability of the index from one lactation to the other was studied on 33 cows and found to be only 0,769. Furthermore, there were no significant differences between 12 sets of supposedly monozygous twins and one set of triplets, but the intra-set differences were significant.

That the repeatability of the front-to-rear index is lower than the estimates of heritability may be due to the fact that a lower limit for

the yield per milking was used in the former calculations, *i. e.* 2 kg milk compared to 4 kg for the heritability estimates in the same breed. It is shown in Tables 3 and 4 that the variation in the indices increases with advancing lactation and decreasing yield. In regard to the twin material it has been pointed out before that many quarters of these cows were damaged by mastitis. The present twin data are, therefore, not suitable for a study of the heritability of the indices of normal udders, and should be disregarded.

The heritability of the front-to-rear index may be estimated according to a third method. *A priori* it would be expected that mastitis, which according to most investigators may show a slight preference for the rear quarters but within the front and rear udder is attacking the glands at random, as well as coincidences in the development of the glands, cause about the same amount of non-genetic variation in the front-to-rear as in the left-to-right index. The variance between cows is for front-to-rear index $37{,}_{39}$ and for the left-to-right index $8{,}_{87}$ (*cf.* Table 5). Considering the latter as an estimate of the non-genetic variance in the front-to-rear index, the heritability of this index may be estimated as $\frac{37{,}_{39} - 8{,}_{87}}{37{,}_{39}} = 0{,}_{763}$, a figure which turns out to be almost identical with the coefficient of repeatability ($0{,}_{769}$) computed from another set of data. Therefore, on the basis of the present investigations the heritability of the relative yield of the front half of the udder (*i. e.* the front-to-rear index) would seem to be about $0{,}_{75}$.

The close agreement between this estimate and the coefficient of repeatability tends to show that the whole genetic variation is additive; no room is left for the effects of dominance or gene interactions. It seems possible that the inheritance of the udder proportions is fairly simple, and not really polygenic as we have tacitly assumed. However, the problem of the mode of inheritance has to be solved by breeding experiments, and will not be discussed here.

There seems to be very good prospects of selection for improved symmetry between the front and rear udder in our dairy breeds, because the total variance in the front-to-rear index is rather large, and the major part of the variance is apparently genetic in origin.

SUMMARY.

An investigation has been made on the relative yield of the different quarters of the udder of the cows in the three Swedish dairy breeds.

The material comprised 591 cows being the progeny of 62 bulls, and contained also 71 dam-daughter pairs. The cows were milked two times each on consecutive days with a special milking machine containing four compartments or sectors, one for each quarter of the udder. Corresponding data were available also for 12 sets of monozygous twins and one set of triplets. In the analysis of the data the following results are obtained.

(1) The left and the right half of the udder produce, on an average, the same amount of milk. The variation in the relative yield of these halves is wholly non-genetic. The major part of this variation is probably caused by clinical or sub-clinical attacks of mastitis, but to some extent it may be caused also by coincidences in the development of the glands.

(2) The front half of the udder produces, on an average, about 41 per cent of the total yield of milk, and the standard deviation of this »symmetry index» is 2 times greater than that of the left-to-right index. The repeatability of the front-to-rear index from one lactation to the other was for 33 cows in the station herd 0,77. On the basis of this figure and the correlation between relatives it is estimated that the heritability of the relative yield of the front half of the udder is about 0,75. Selection for improved udder symmetry should therefore be successful.

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PROGENY TESTS WITH FOREST TREES

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IN plant breeding, progeny tests play a very important part. The reason for this is the fact that a progeny from a given specimen generally provide far better information with respect to those hereditary properties of the specimen which are of economic value than does the specimen itself. Under cultivation many characteristics are important which cannot possibly be judged from one single specimen. In forestry, the yielding capacity per area unit is a very significant varietal quality. Therefore little is generally gained by selecting trees whose offspring are very good when planted solitarily, if the output per area unit is low.

Somewhat schematically, the breeding work may be divided into two parts, one comprising the breeding of new varieties and the other the testing of these new varieties. To distinguish between the experiments conducted in the two parts of the breeding work, the experiments of the first part are in the following referred to as progeny tests, whereas the others are called variety trials.

In recent years, the planning, execution and analysis of variety trials has become a highly developed technique, so that today the method of conducting such experiments in order to obtain the best results, is quite well known. However, less is known of the progeny tests.

The results of the progeny tests may give a good expression of the properties of the progeny tested. But in many cases we are especially interested to get information of the *parents* selected, because we wish to know if these parents are to be used in further breeding work or not. With this end in view, the experiments ought to be planned in such a manner that the greatest possible correlation is obtained between the characteristics of the progeny and the genotypes of the parents.

The variety trials, on the other hand, shall yield information regarding existing differences among the varieties. Hence, the problems presented by this case are somewhat different from the former. When a variety trial has been completed, it is generally analysed statistically. The varieties which are significantly better than a given standard are recommended for further cultivation, while the other varieties are discarded or kept for continued breeding.

In progeny tests, the treatment may be of a different nature. This is particularly true when the question is one of testing basic breeding material. Then it often happens that no marked differences are found between the different strains. Yet, for practical reasons, it would be impossible to continue breeding work with all the strains included, so the poorest are often discarded even though no significant differences appear from the experiments. It is, moreover, known that even when rather great differences actually exist among the strains, these differences are not always revealed in the experiments in such a manner as to make them »statistically» significant.

In breeding work one is often faced with a choice of various methods of progeny testing. For example, if certain definite seed trees have been selected for testing, such testing may be based upon open or artificial pollination. The first method will usually be the cheaper one, but is, on the other hand, not quite so effective as the second method, although little is known about the degree of inferiority.

For the purpose of gaining some basis for such decisions, three different types of progeny tests are here presented, a discussion of which in greater detail seems justified.

(I) Definite seed trees are selected. The progeny of these trees are planted in experimental plots, each plot containing *m progeny from each seed tree*, the experiment being carried out in *n* replications.

(II) In a number of stands, *t seed trees are selected from each stand*. The progeny of *each seed tree* are planted out in experimental plots, with *m* progeny per plot, and in *n* replications.

(III) The progeny of a number of seed trees from different stands (provenances) are set out in experiments with *m progeny from each stand* (*provenance*) in each plot, and in *n* replications.

For these cases, I have developed formulae which express the relationship between the records of the offspring and the genotypical value of the seed tree. The calculation of these formulae is most easily undertaken by means of »path coefficients» (WRIGHT, 1934), but they may also be developed quite readily in the usual manner.

RELATIONSHIP BETWEEN THE GENOTYPICAL VALUE OF THE SEED TREE AND THE RECORDS OF THE OFFSPRING.

The formulae are based upon certain assumptions. It is supposed that random matings occur among the trees, also, that the environment has an additive effect upon the genotype, so that the absolute effect of

one particular environmental influence is equal for all the different genotypes.

LUSH (1935) gives the following definition of the genotype (the sense in which it is used in the formulae): »'Genotype' is obtained by assigning each gene such a value that the simple sum of all the genes in each individual will give the 'genotype' for that individual subject to the 'least squares rule', that (1) the average genotypic value of the population shall coincide with its average phenotypic value and (2) the sum of the squares of the individual deviations of 'genotype' from 'phenotype' shall be a minimum.» In selection breeding, the genotypical value thus assessed is of special importance.

The following symbols are used:

r_{gg} =the correlation between the genotypes of the two parents.
r_{oo} =the total correlation between individual records within the same plot.
r_{ee} =the correlation between individual records within the same plot, as are caused by environment.
h^2 =the hereditary part of the total variance.
e^2 =that part of the total variance which is due to varying environmental conditions.
g^2 =that part of h^2 which may be expressed by the additive gene effect.
m =number of trees (progeny) per plot.
n =number of replications.

For the purpose of illustrating the calculation of such formulae as given below, without using »path coefficients», let us suppose that the correlation is to be found between the mean value of the records of one particular character of m progeny from a given seed tree, and the genotypical value of the seed tree.

According to the presumptions made, random matings occur whitin each stand. The correlation r between the record of an individual progeny and the genotypical value of the seed tree is $\frac{hg}{2}$ when only one stand is considered. If seed trees from several stands are used, the correlation will be

$$\frac{hg}{2}(1+r_{gg}).$$

When the progeny are planted for experiments, we assume for a moment that all offspring from one seed tree are set in the same plot.

Within one experimental field, the soil quality will nearly always vary somewhat from one plot to another, with the result that the plant records from one and the same plot become correlated; the records will also be correlated because of the plants being siblings or half-siblings.

The above-mentioned correlation will be

$$\frac{\operatorname{cov}\left[\frac{c_1+c_2+..+c_m}{m},\ G\right]}{\sqrt{\operatorname{var}\left[\frac{c_1+c_2+..+c_m}{m}\right]\operatorname{var} G}}=\frac{hg}{2}(1+r_{gg})\sqrt{\frac{m}{1+(m\div 1)r_{oo}}}$$

where c_i is the record of the progeny i and G is the genotypical value of the seed tree.

The formulae given below may be developed in the same manner.

In the following, the mean value of the records of a definite character for $m \cdot n$ progeny is called the mean value.

Case I. — With open pollination, the correlation between the mean value and the genotypical value of the seed parent is

$$r_1=\frac{hg}{2}(1+r_{gg})\sqrt{\frac{mn}{1+(m\div 1)r_{oo}+(n\div 1)m\,\frac{h^2g^2}{4}(1+3r_{gg})}} \tag{1}$$

$$\text{where } r_{oo}=r_{ee}e^2+\frac{h^2g^2}{4}(1+3r_{gg}) \tag{2}$$

With artificial pollination, it is possible to choose the male parent to be used in the crosses. The most extreme cases is found when the genotypical value of the male parent is known. The correlation between the mean value of $m \cdot n$ progeny and the genotypical value of the female parent is

$$r_2=\frac{hg}{2}\sqrt{\frac{mn}{1+(m\div 1)r_{oo}+[(n\div 1)m\div 1]\frac{h^2g^2}{4}}} \tag{3}$$

$$\text{where } r_{oo}=r_{ee}e^2+\frac{h^2g^2}{4} \tag{4}$$

With open pollination, the regression for future progeny on the mean value of the progeny from one seed parent is

$$b_{FM}=\frac{r^2{}_1(1+3r_{gg})}{(1+r_{gg})^2} \tag{5}$$

(The correlation coefficient depends upon the number of future offspring. This is the reason for using the regression coefficient rather than the correlation coefficient when treating the relationship between future progeny and the progeny mean in the experiment.)

Case II. — The genotypical value of a seed tree from a definite stand is to be determined. This case presents the possibility of estimating the genotypical value of the female parent by means of a multiple linear regression equation including the progeny mean x and the average of the t other progeny means from the same stand, y. The multiple correlation coefficient is:

$$r_3 = \sqrt{\frac{r^2_{gx} + r^2_{gy} - 2r_{gx}r_{gy}r_{xy}}{1 - r^2_{xy}}} \qquad (6)$$

where r_{gx} is the correlation between the genotypical value of the seed tree and the mean value of the progeny, r_{gy} is the correlation between the genotypical value of the seed tree and the average of the t other progeny means from the same stand, and r_{xy} is the correlation between the progeny mean from the seed tree, and the average of the t other progeny means from the same stand.

Case III. — The correlation between the mean value of the progeny and the mean genotypical value of the selected seed trees from one stand is computed in a similar way as the formulae given above (Table 4).

The regression for future offspring from the same seed parent on the mean value in the experiment is given in Table 5.

DISCUSSION.

All the formulae given above are based upon the assumption of random mating. When this condition is not fulfilled, all the correlation and regression coefficients will be somewhat lower than indicated. The formulae may, however, be extended as to cover these cases also. This seems to be an important point, because, in practice, there will never be just a random mating among the forest trees.

It has previously been mentioned that the results are based upon the presumption that the environment has an additive effect upon the genotype in such a manner that *one environmental effect has an equal absolute effect upon the various genotypes.* This condition is not likely fulfilled and the formulae will therefore give somewhat fallacious re-

sults, but on the whole the matter is largely one of using a suitable scale for the records of the characters measured. If, for example, the height increment of the trees is to be measured, it is possible that an increment measurement in centimetres does not meet the above condition. There are, however, many other scales which may be applied, and it seems to be possible in the most cases to find such a scale that the environmental and the gene effects are approximately additive. The difficulty is that it is not possible as yet to say which scale is to be recommended for a given case. This can only be determined after analysing a series of similar cases planned to clarify this problem.

It seems reasonable to discuss in some detail the meaning of the separate parts of the formulae.

The correlation between the genotypical values of the parents, r_{gg}, may be defined as follows: If the total hereditary variance is σ^2_t and the variance for the mean genotypical values of the stands is σ^2_m, then

$$r_{gg} = \frac{\sigma^2_m}{\sigma^2_t}$$

In all experiments a certain variation will exist among the individual specimens. When considering one particular experiment, the total variance may be divided into two parts, σ^2_e and σ^2_h, where σ^2_e expresses the variance caused by the environment, and σ^2_h expresses the variance due to hereditary differences among the individuals. In experiments laid out in randomized blocks (and similar designs), a considerable portion of σ^2_e is to be found »between blocks». Within the blocks the variance due to environmental conditions σ^2_{eb}, is much lower than σ^2_e, hence in such experiments h^2 is

$$\frac{\sigma^2_h}{\sigma^2_{eb} + \sigma^2_h}$$

and e^2 is

$$\frac{\sigma^2_{eb}}{\sigma^2_h + \sigma^2_{eb}}$$

In the calculation of the tables, $h^2g^2+e^2$ is set at 1. This means that the calculations are based on the presumption that the gene effects are additive exclusively, with no interaction taking place between the genes (dominance, epistasis).

Introducing a coefficient d^2 so that $h^2g^2+h^2d^2+e^2=1$, and assuming that d^2 is of the order, *e. g.*, $^1/_2\, g^2$, it is seen that this leads to only small modifications of the figures in the tables.

TABLE 1. *The correlation coefficients (in per cent) for the mean value of m progeny in 4 replications, and the genotypical value of the seed parent (formula 1). The bottom line applies to artificial pollination (formula 3).*

	$m = 4$								$m = 50$							
	$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$				$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$			
r_{gg}/e^2	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8
0	88	83	75	60	85	76	64	47	97	93	86	73	91	80	67	49
0,2	88	85	78	66	86	79	69	53	93	91	86	76	88	82	71	55
0,4	90	87	82	71	88	83	74	59	93	91	88	80	90	85	76	60
0,6	93	90	86	76	91	86	79	64	95	93	90	84	92	88	80	65
A. p.	90	85	76	61	86	77	65	47	97	93	87	73	91	80	67	49

TABLE 2. *The regression coefficient (in per cent) for future offspring on the mean value of m offspring from the same seed tree in 4 replications (formula 5).*

	$m = 4$								$m = 50$							
	$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$				$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$			
r_{gg}/e^2	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8
0	78	69	56	36	71	57	41	22	94	86	75	53	82	64	45	24
0,2	86	79	68	48	82	69	53	31	96	91	83	65	88	74	57	33
0,4	91	85	76	56	87	76	61	39	97	93	87	72	91	80	64	41
0,6	94	89	81	63	91	81	67	45	98	95	89	76	93	84	70	46

TABLE 3. *The multiple correlation coefficient (in per cent) (formula 6); r_{gg} is set at 0,2.*

	$m = 4$								$m = 50$							
	$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$				$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$			
t/e^2	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8
2	89	85	78	66	86	79	69	54	95	92	87	76	90	82	72	56
5	89	85	78	66	86	79	69	56	96	92	87	76	91	82	72	57

TABLE 4. *The correlation coefficient (in per cent) for the mean value of m offspring in 4 replications and the mean genotypical value of seed trees from one stand (provenance).*

	$m = 4$								$m = 50$							
	$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$				$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$			
r_{gg}/e^2	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8
0	42	35	27	18	37	28	20	13	26	17	12	8	15	9	6	4
0,2	86	80	71	56	82	72	60	43	96	91	84	69	89	77	63	45
0,4	93	89	83	69	90	83	73	56	98	95	91	80	94	86	75	58
0,6	96	93	88	77	94	88	80	64	99	97	94	86	96	90	81	65

TABLE 5. *The regression coefficient (in per cent) for future offspring from the seed trees of a given stand, on the mean value of m offspring in 4 replications, from the same trees.*

	$m = 4$								$m = 50$							
	$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$				$r_{ee} = 0{,}2$				$r_{ee} = 0{,}8$			
r_{gg}/e^2	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8	0,2	0,4	0,6	0,8
0	18	12	7	3	14	8	4	2	7	3	1	1	2	1	0,4	0,2
0,2 ...	77	67	53	33	70	54	37	19	92	84	71	48	79	59	40	20
0,4 ...	89	81	70	49	83	71	54	32	96	91	83	65	88	75	57	33
0,6 ...	93	88	78	59	89	79	64	41	98	94	88	73	92	82	66	43

It appears from the tables that if 4 replications are used in the experiments, an increase in the number of individuals per plot from 4 to 50 affects the accuracy (accuracy being measured by the correlation coefficient) of the experiment only slightly.

For the same number of individuals per plot, the accuracy of the experiment decreases only slightly with an increase in r_{ee} from 0,2 to 0,8.

It appears from formulae (1) and (3) (Table 1) how relatively unimportant it is to know the genotypical value of the male parent. This is, of course, related to the presumption of random matings. Frequently it is possible to use homozygous male parents for certain genetic investigations, which is always very advantageous. At the present stage of forest tree breeding, however, the male parent material available for artificial pollination must be considered just as heterozygous as the female parent material, and the formula is developed for this case.

Table 3 shows formula (6) for two values of t ($r_{gg}=0{,}2$). It is seen that for the determination of a certain seed tree, the progeny of another tree from the same stand provide relatively little information which is not available from the offspring of the tree itself.

Provenance experiments may be designed in a similar way as in case II. But if this is done, the mean of the progeny means of the same provenance will have a larger mean error than when the experiments are designed as in case III.

The magnitude of r_{ee} and r_{gg} varies from one experiment to another. The larger the existing differences between the stands are, the larger is r_{gg}. It will, however, seldom be larger than 0,4. r_{ee} increases with increasing differences in soil quality between the plots.

While studying at Brunsberg, one of the stations belonging to the Swedish Forest Tree Breeding Institute, the leader of the station, Dr. ENAR ANDERSSON, was kind enough to give me some material for further examination.

Four experiments are used, two with spruce and two with pine. The experiments are still in a very early stage as they were started only 3—4 years ago.

In the spruce experiments r_{gg} is 0,36 and 0,11, respectively, the corresponding values of the pine experiments being 0,19 and 0,05.

In one spruce experiment $r_{ee}e^2$ is of the order of magnitude of 0,03 (with 49 plants per plot), whereas it is 0,13 in one pine experiment (with the same number of plants per plot). These values were not computed for the two other experiments.

The progeny tests may be used to test whether the difference between two varieties (seed trees) is significant. Furthermore, they may provide some basis for estimating the magnitude of such differences. It might be of some interest to see how the afore-mentioned coefficients enter the picture when the problems are viewed from this angle.

Let us suppose that a progeny test has been made with some selected seed trees from one stand. The experiment is laid out according to an ordinary experimental design. The experiment is subjected to a statistical analysis, and a t-test is performed between two varieties (progeny means). The t-test tests the hypothesis that the two means are equal.

It may be distinguished between two eventualities:

(1) Actually, the means are equal. Nevertheless, a certain probability α exists of a significant t value, meaning that if the experiment were to be repeated an infinite number of times under the same conditions, a significant t value would be found in α of all cases. (Error of the first kind.)

(2) Actually, the means are different. The probability of a non-significant t value is β, in the same sense as stated above. (Error of the second kind.)

Standard deviation of a single offspring in the experiment is called σ. The true difference between the genotypical values of the two seed parents (whose offspring are entered into the experiment) is called Δ. Then the true difference between the progeny means will be $\frac{\Delta}{2}$ on an average. It is possible to demonstrate that β depends upon $\frac{\Delta}{2\sqrt{2}\varepsilon}$ where ε is the standard error (NEYMAN *et al.*, 1935).

$$\frac{\Delta}{2\sqrt{2}\,\varepsilon} = \frac{\Delta\sqrt{mn}}{\sigma 2\sqrt{2}\sqrt{1+(m\div 1)r_{ee}e^2}}$$

Mention has already been made of the fact that the experiment may provide clues towards estimating differences between the varieties (seed trees) which are entered into the experiments. Hence the presentation is the same as in stock breeding, where »sire indices» are determined for such characters which cannot possibly be registered in the male.

It is supposed that one of the parents (below called the »mother» in order to facilitate a comparison with the problem in stock breeding) equals the average of the population. Perhaps the most widely used index-formula is that of HANSSON-YAPPS: If the index value of the father is called z and the mean value of the records of the offspring is called y, then

$$z=2y$$

both z and y being measured from their means. Whether this method is suitable for the calculation of the indices depends upon the purpose for which the values are to be used.

The cases of most vital importance are, perhaps:

(1) By means of the index value, an estimation is to be made of the future offspring of the father in question.

(2) The index figure shall express the hereditary characteristics of the father (his »breeding value»).

These two problems are rather different and should be kept apart. If the progeny mean of an individual deviates δ from the population mean, the best estimate for the future offspring from the same individual (assuming random mating) is given by

$$b\delta$$

where b is the regression coefficient for future offspring on the offspring in the experiments. This would correspond to an index value equal to $2b\delta$ for the father, when using a prediction formula of the type $y_p=0{,}5$ (index value).

Only in rare cases should k be set at 2 in the general formula $z=ky$ (as in the HANSSON-YAPPS' index-formula), when the problems are posed as mentioned under point 2, since too much weight would then be placed upon the lesser variations in the progeny means.

When we have observations from one single experiment only, the value given to k is of minor importance, because all values of k will give the same ranking of the fathers. In those cases where there are observations from several experiments, however, a good estimate of the index value of a father seems to be

$$\frac{w_1^2\delta_1 + w_2^2\delta_2 + \ldots + w_n^2\delta_n}{\sum_i w^2_i} = I$$

where I is the index value, δ_i is the deviation of the progeny mean from the total mean in experiment i, and w_i is the reciprocal of the standard error in experiment i.

SUMMARY.

Different formulae have been given showing (a) the relationship between the genotypical value of a parent (or the mean value of several parents), and the mean of m offspring in n replications, (b) the relationship between future offspring and the mean of the offspring in the experiments.

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CYTOGENETIC INTRASPECIFIC STUDIES IN LAMIUM. I

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I. INTRODUCTION.

AS previously reported (BERNSTRÖM, 1941, 1944), an investigation into the cytogenetic relationships between the North-European annual *Lamium* species has been made, the results of which are as yet only pointed to (BERNSTRÖM, 1949) and which are to be dealt with in a future paper. In order to give as firm a basis to the investigation as is practicable, attempts have been made to collect a truly representative material of all species, each being represented by several pure lines collected from parts differing as widely as possible within its distribution area.

For the same purpose it was also considered desirable to obtain some knowledge about the cytogenetic properties of the pure species themselves. As a rule, it has not been a question of comprehensive investigations, but rather of preliminary ones, which could be carried out within the limits of accommodation at disposal and the call of more important work.

Thus, studies have been made on the fertility and, where it has been desirable and possible, the meiosis both of the pure species concerned and of intraspecific interlinear hybrids produced in each species. Attention has also been paid to the morphology of the hybrids, especially with a view to establishing the possible occurrence of heterosis. These studies, which were commenced in 1943, gradually tended to become rather extensive, and as they now cover a considerable amount of experience, it is felt they are worth publishing. The object of some of the experiments will be further discussed under the various species.

The species principally studied are *Lamium purpureum* L., *L. amplexicaule* L., *L. hybridum* VILL., and *L. intermedium* FR. They belong to the group of annual species of the section Pollichia of the genus, to which group also belong some five South-European—West-Asiatic species, comprising *L. bifidum* CYR., *L. macrodon* BOISS. et HAUSSKN. and some species which, from a morphological point of view, seem to

be nearest related to *L. amplexicaule* and which are evidently little known systematically (see below).

L. purpureum, *L. amplexicaule*, *L. hybridum*, and *L. intermedium* are good species, well separated from each other morphologically. Extensive studies on dried material from the herbaria of Lund, Upsala, Stockholm, Gothenburg, Copenhagen, and Helsingfors have shown that there are hardly any specimens among the species concerned which are difficult to determine taxonomically. In uncertain cases it is always a question of young or abnormally grown individuals. Reports of intermediary types between *L. hybridum* and *L. intermedium* probably refer to such specimens which are less suitable for taxonomical determination.

L. hybridum and *L. intermedium* are, each in its way, more or less intermediary, from the morphological point of view, between *L. purpureum* and *L. amplexicaule* and, for that reason, have often been regarded as F_1 hybrids or descendants of hybrids between the two last-mentioned species. Earlier studies of the constancy and descent of *L. hybridum* and *L. intermedium* have been made by LINDGREN (1841), FOCKE (1881), DANSER (1925), MÜNTZING (1926), and JÖRGENSEN (1927).

The authors mentioned above establish the fact that offspring from *L. hybridum* and *L. intermedium* remain constant. DANSER points out that the seed-setting of both species is good. MÜNTZING and JÖRGENSEN have tried to hybridize the four species concerned and have found that it is only possible to obtain hybrids between *L. hybridum* and *L. amplexicaule*, and that these hybrids are sterile and do not conform morphologically to any one of the other two species. JÖRGENSEN has revealed that *L. purpureum* and *L. amplexicaule* are diploid, having $2n=18$, while *L. hybridum* and *L. intermedium* are tetraploid ($2n=36$).

GARCIA (1947), in a treatise in Portuguese, describes a form of *Lamium* discovered in one place in Portugal as a special species which — if we understand him correctly — is the result of a crossing between *L. amplexicaule* and *L. purpureum*. Tests have shown that it is fertile and constant. No crossing experiments have yet been made, nor has it been studied cytologically; and as it has been impossible to obtain seed from this species, it must, for the present, be left for what it is worth.

The original distribution of *L. amplexicaule* is considered to be Central and Western Asia and the Mediterranean, but the species is now distributed with the culture all over Europe and has also spread to the other continents (HEGI, 1927; HÖCK, 1900). *L. purpureum* is found in practically the whole of Europe and in large parts of Northern and Central Asia; its original distribution area seems difficult to ascertain.

L. hybridum occurs in Northern and Central Europe and seems to be most common in the South and South-East Baltic provinces, while *L. intermedium*, occurring perhaps more sparsely within its distribution area than the other species in their respective areas, is geographically the most restricted of the species under discussion. Its distribution area comprises Iceland and Great Britain in the West, Western Russia in the East, Holland, Central Germany and the East of Prussia in the South, and is bordered by the Arctic circle to the North.

II. MATERIAL AND METHODS.

1. MATERIAL.

Half or more of the material studied was collected in different parts of Sweden, the remainder having been obtained from abroad. Owing to difficulties brought about by the war, the foreign material is represented by a much smaller group than would have been desirable. Some lines have been obtained from dried specimens in herbaria, seeds up to 11 years old having been saved. In the detailed account of the different species, particulars about the material and its origin will be given.

Because all the species studied are autogamous, the experimental material consists of pure lines. To each line used in the experiments has been affixed its own number, preceded by the initial of the species' name concerned (for example, *a*9, *h*37).

2. METHODS.

In order to avoid, as far as possible, unfavourable effects from the weather, which might easily be severe to a plant material as weak as *Lamium*, the cultures were kept in unheated greenhouses and frames. This has made possible a good growth of the plants, but for want of space it has also brought about the great disadvantage of enforced limitation, often very considerable, in the number of plants cultivated. As a rule, pots with an upper exterior diameter of about 14,5 cm. have been used. The pots were usually placed close to each other, a distance of about 15 cm. between the plants being employed. In 1949, a distance of about 20 cm. was kept between the plants.

Thanks to the rapid development of the species studied, it was possible to cultivate two generations a year in 1943—1945. The first sowing was then performed between April 10th and 14th, and the

flowering was in progress from the last week of May to the latter part of June, or longer for the slower types. The second generation was sown between July 22nd and 28th, and its flowering took place from the last days of August till far into October, or even into November for the slower part of the material. As a rule, all the species thrive in the late autumn as well as in the summer and they set seed almost as well, too. The greatest disadvantage with autumn generations is an inferior result from cross-pollinations (see below, and unpubl.). During the years 1946—1950 the plant material flowered in July and August.

The year of each plant generation is indicated by the last two figures of its number, and in cases of two generations a year, the number of the generation is given after that of the year (for example, *a*9—44—2).

Usually, the germination of both newly harvested and older seeds of all the species progresses in a very unsatisfactory and uneven manner. From 1944 this difficulty was overcome by removing the seed-coats by hand (the method suggested by MÜNTZING; *cf.* MÜNTZING, 1930). The seeds are kept moist for 2—4 days and the embryos are then laid free with the aid of a lancet and placed on a moist paper under small bell-glasses. Treated in this way the seedlings develop rapidly, usually being able to be pricked out in pots within 5—7 days. However, embryos from winter-annual and similar types (*cf.* MÜNTZING, 1932 b) refuse to germinate even after this treatment, or germinate very slowly and in small numbers. In order to enable direct comparisons of the rate of development, time of flowering *etc.*, those numbers which were to be compared were treated simultaneously. As a rule, the removal of seed-coats from the seeds of a plant generation seldom took more than 3 days.

For determination of somatic chromosome numbers, root-tips were fixed in Navashin's solution as modified by MÜNTZING (1932 c). They were cut into sections 12 μ thick and stained with gentian or crystal violet. For investigations of the meiotic divisions, fixatives with osmic acid must be used; even if the fixation of the chromosomes in the different stages of the meiosis is mostly unsatisfactory with these fixatives also, they nevertheless give better results than Navashin's solution. I-M was found to be the stage which most readily leant itself to fixation, but, as a rule, a considerable amount of fixed material was required to obtain even a few slides suitable for analysis. Most of the fixations fit for use were obtained by pre-treatment in Carnoy 6 : 3 : 1 for one minute, replaced by either Fleming-Benda or Strong Fleming (DARLING-

TON and LA COUR, 1942), the buds then being subjected to vacuum. When fixed in Strong Fleming, the chromosomes are less contracted than when fixed in Fleming-Benda, the configurations then being less detailed. The buds were cut into sections 17—18 μ thick, and stained in crystal violet. Because of the very small size of the buds and the rather inconsiderable number of PMC's in each anther, no smearing technique was used.

The percentage of good pollen grains was studied in a mixture of aceto-carmine and glycerine (1 : 1). At least 200 grains, and as a rule many more, were counted in each slide. Grains, well stained and filled, were counted as fertile, while poorly filled, or slightly coloured and colourless, were counted as sterile. With pollen fertility is meant the percentage of good pollen grains.

As previously related (BERNSTRÖM, 1950 a), the degree of seed-setting was determined on the 2(—3) whorls situated at the lowest flowering nodes of the stem. At least 20 calyces and, as a rule, still more were examined. They were studied after the dropping of the ripe seeds, it being then possible to count the undeveloped ovules *in situ*. The estimation of the degree of seed fertility was based on the fact that the gynaecia always consist of four ovules. The seed fertility is expressed in percentage of ripe seed.

By cross-pollination of the buds, emasculation and pollination were made in succession and at all times of the day. Buds were used which should have opened within 24 hours.

Seed-setting by the cross-pollination method used exceeded 70 % in the best cases, *viz.* in *L. purpureum* in the summer; *cf.* Table 1. In this, only the years 1943—1945 are included, when two plant generations a year were cultivated.

It will be clearly seen from Table 1 that the results of cross-pollination are dependent on the season, summer seed-setting being superior to that of the autumn. Experience of interspecific cross-pollination in *Lamium* (unpubl.) confirms the results set out in Table 1; often more difficult of realization than the intraspecific cross-pollinations, they clearly demonstrate, in particular, the advantage of hot and dry over cool and moist weather for seed-setting. The dependence of successful crossing on the climatic conditions is well noticed, as demonstrated, *e. g.*, by LAMM (1945, p. 9) in *Solanum*.

It is apparent from Table 1, also, that seed-setting after cross-pollination is somewhat higher in the diploids than in the tetraploids, as is also the case in seed-setting after self-pollination.

TABLE 1. *The results of cross pollinations within species of Lamium.*

Cross combinations	Season	Number of flowers pollinated	Number of seeds obtained	Number of plants obtained	Number of hybrids obtained	% seed-setting of hybrid seeds	Number of cross combinations tried	Number of lines used ♀	Number of lines used ♂	Years
2n purp. × *2n purp.*	summer	221	657	133 [1]	130	72,6	15	6	6	44—45
	autumn	275	240	129 [1]	126	21,3	14	9	7	43—45
2n amplex. × *2n amplex.*	summer	137	389	128 [1]	122	67,6	12	4	6	44—45
	autumn	29	74	31 [1]	31	63,8	3	2	2	43
hybridum × *hybridum*.....	summer	141	329	74 [1]	72	56,8	5	3	5	44—45
	autumn	160	57	33 [1]	32	8,6	7	5	4	44
interm. × *interm.*	summer	110	283	56 [1]	56 [2]	64,3	5	4	3	44—45
	autumn	52	49	31 [1]	31 [2]	23,6	3	2	2	44

[1] The number of plants cultivated was lower than that of available seedlings, in one or several generations.
[2] It was impossible to decide if the plant material consisted solely of hybrids.

III. FLOWER BIOLOGY.

The flower biology of *L. amplexicaule* and, to some extent, that of *L. purpureum* and *L. hybridum*, has been treated earlier (BERNSTRÖM, 1950 a, b). Of the species studied, chasmogamic flowers regularly occur only in *L. purpureum*, *L. hybridum* and *L. intermedium*. In *L. amplexicaule* the chasmogamic flowers will sometimes be lacking completely, in contrast to the cleistogamic ones which always occur (*cf.* BERNSTRÖM, 1950 b). According to some earlier reports, frequently quoted, the flowers of *L. purpureum* are extensively pollinated by honey- and pollen-collecting insects, self-pollination only taking place in the absence of insects; accordingly, this species at least would be partially allogamous (KNUTH, 1899, 1905; v. KIRCHNER, 1911; HEGI, 1927). As stated earlier (BERNSTRÖM, 1950 a, b), self-fertilization of chasmogamic flowers of the species studied is mostly effected during the expansion of the flowers and sometimes afterwards, by the aid of external influences, as the anthers take up positions close to either side of the stigma. Consequently, as a rule, insects will effect only renewed pollinations.

According to investigations on material cultivated in greenhouses, about 10 % of the flowers of *L. amplexicaule* fail to self-pollinate, while the number of unpollinated flowers in the other species rarely exceeds 5 %, being mostly about 1—2 %. Only towards the end of the flowering season, when the size of the flowers diminishes more or less (*cf.* below, *L. hybridum*), the number of unpollinated flowers easily increases; this was found especially in *L. hybridum* in which up to 10 % of such flowers were observed. The increase is caused by a greater variation in the length of the stigmas proportionate to that of the anthers.

The existence of certain possibilities of cross-pollination by insects visiting unpollinated flowers raised the question as to which species are visited by honey- and pollen-collecting insects able to effect cross-pollination; also the subsidiary question of how often they are visited. It became evident that the flowers of the different species differ widely in the attraction they exercise on insects. During the summer, with its vast range of selection, bees and humble-bees show preference for *L. purpureum*. This becomes particularly obvious when the material is cultivated in open frames. *L. hybridum* and *L. intermedium* are visited less often than *L. purpureum*, and *L. amplexicaule* even more rarely still. During the autumn months August—October, the range of choice

is narrowed and the insects become less fastidious, visiting all open flowers, though *L. purpureum* is still clearly preferred.

In consequence of these facts occasional cases of vicinism are to be expected, and the following hybrids have actually been found:

(1) In *a*9—44—2 three plants occurred, the seed-fertility values of which were 42, 5 and 53, respectively, while those of their sister plants were about 90 %; the three plants concerned were somewhat stronger in growth, too, but otherwise fairly similar to *a*9. Reduced seed fertility and vigorous growth are very often characteristic of intraspecific hybrids in *L. amplexicaule*. Offspring were raised from the two most sterile plants and also from a fully fertile and normal plant of *a*9. The following average values were obtained:

Parent	Seed-setting of the parent	Average of seed-setting of the offspring	Average of pollen fertility of the offspring	n
*a*9b$_5$—44—2	42 %	66,7 %	88,3	7
*a*9b$_5$—44—2	5 »	45,0 »	52,3	7
*a*9—44—2	98 »	89,8 »	98,6	14

The quotient between the mean squares of »between offspring» and »within offspring» in an analysis of variance of the values of seed-setting mentioned above was 57,6 ($P < 0{,}001$). Though values for seed-setting are always less reliable than those for pollen quality, the mean of which always exceeds 97 % in the pure lines of *L. amplexicaule*, at least the two plants analysed must be considered to be spontaneous intraspecific hybrids. From what is said below (p. 179) about the border between full fertility and partial sterility, as estimated on the pollen, it is apparent that the averages for pollen fertility confirm the results.

(2) In *p*26—46 a plant with red pollen but with some typical features of *p*26 occurred. *p*26 has yellow pollen, which is recessive to red. In *p*53—48 one plant appeared with the normal purplish-red colour of flowers characteristic of *L. purpureum*, instead of the rosy which characterizes *p*53; the flower-colour of *p*53 is recessive to purplish-red.

(3) In *h*18—44—2 one plant was a hybrid with tetraploid *L. purpureum*. Morphologically, it corresponded completely with the hybrids between *L. hybridum* and the tetraploid *purpureum* lines *p*6 and *p*7, these hybrids being characterized, above all, by their dwarfishness. In the first generation of 1944, when the hybrid seed was obtained, a

distance of about 1 metre separated *h*18 from *p*6 and *p*7, the plants being placed in open frames.

A hybrid between the same two species was found in 1947 in the tetraploid line *p*42; thus, *L. purpureum* had acted as mother in this case, which is remarkable, as it is rather difficult to get hybrids by cross-pollination in this direction.

It must be added, however, that it is not outside the bounds of possibility that more cases of vicinism have occurred, since some lines of *L. purpureum* and *L. hybridum* resemble each other so closely as to permit, perhaps, occasional intraspecific hybrids to escape attention. In *L. intermedium*, all the lines cultivated were almost completely identical, possible cases of vicinism thus being quite hidden.

From what has been said above, however, and considering that hundreds of plants were cultivated in every species, it is evident that the species studied are very strongly autogamous. Thus, isolation by bagging to keep the lines pure was not required at all.

The cleistogamic flowers of *L. amplexicaule* have been treated earlier (BERNSTRÖM, 1950 b). HEGI (1927) and others point out that cleistogamic flowers also occur in *L. purpureum*. It may be added, from my own experience, that the flowers of *L. intermedium* and, to an even greater degree, those of *L. hybridum* are sometimes closed. Some lines, especially in *L. hybridum*, are more apt than others to behave in that way (see below), the closed flowering predominating in the autumn, as a regularly occurring phenomenon. Often the flowers do not open in *L. hybridum*, even in the summer, towards the end of flowering, especially when the predominant weather is not at its best. Pseudo-cleistogamy is undoubtedly the most correct way of describing this type of closed flowering, the term including both GOEBEL's (1904) signification of retarded opening (while true cleistogamy manifests itself as retarded development) and LINDMAN's (1908) opinion that cleistogamy is a phenomenon of prematurity, as opposed to chasmogamy which would, and obviously should, cover pseudo-cleistogamic flowers. Cleistogamic flowers of *L. amplexicaule*, and other species, are generally cleistoantheric, while the anthers of pseudo-cleistogamic flowers open and let their pollen fall on the stigma.

The seed-setting of pseudo-cleistogamic flowers is often somewhat inferior to that of the open flowers. In estimating the seed-setting of the autumn material this, however, is irrelevant, for the seed-setting is determined by the lowest flower whorls, which are the first to flower and which also have the best bloom development.

IV. EXPERIMENTAL.

1. L. AMPLEXICAULE.

The following lines of *L. amplexicaule* were used:

*a*8 In the neighbourhood of Gothenburg, Sweden. A comparatively low-growing line, with large, horizontally placed leaves with characteristic, deeply cut segments. Abundant chasmogamic flowering.

*a*9 From Svalöf, Sweden. A fairly tall but delicate line, with rounded-lobed leaves. Fair chasmogamic flowering.

*a*10 From Svalöf. Very tall, with very few chasmogamic flowers.

*a*12 From Coimbra, Portugal. Easily recognized because of its multi-lobed and wavy-edged leaves which are of a dark green colour.

*a*13 From Bucharest, Rumania. Leaves of a pronounced kidney shape, dark green in colour. Fairly rich chasmogamic flowering.

*a*32 From Hälsingborg, Sweden. This line is sensitive to its environment, varying considerably in its plant size from generation to generation. Characteristically green stem.

*a*72 From Siena, Italy. Rather similar to *a*8, but the middle lobe of its leaf is characteristic in shape and the flowers present a different pattern.

*a*78 From Lund, Sweden. Resembles *a*13, but differs in several distinct minor features.

*a*86 From Zahleh, Syria. The flowers are pure red, the plants a fairly light green. Abundant chasmogamic flowering.

*a*88 From Zahleh, Syria. Tall line with unusually big, straight sepals. Very meager chasmogamic flowering.

*a*106 From Szeged, Hungary. Characteristic hand-lobed leaves with a pronounced middle lobe.

*a*108 From Rouen, France. Recognizable by its flower pattern.

*a*109 From Catania, Sicily. Unusually shallow-lobed leaf. Light yellow pollen; in the other lines orange-red.

The pure lines of *L. amplexicaule* dealt with above are all summer-annuals, in the same way that MÜNTZING (1932 b) has shown pure lines of *L. purpureum* frequently to be summer-annuals and as has been the case with a great number of other samples of *L. amplexicaule* received from different parts of Europe but not used in the present experiments. Three samples received under the name of *L. amplexicaule* from Hungary, Southern Russia and Turkey, respectively, refused to

flower the same year they were sown, but only developed into thick bushes with multi-internodical sterile branches; it is possible that these samples represent winter-annual types of *L. amplexicaule*. The shape of the leaves which were very deeply laciniated, resembled those of herbaria specimens from the Ukraine, which are sometimes termed *L. amplexicaule* var. *orientale* PACZ.

The varieties mentioned resembled, in their leaf shape, one line *a*83, which was used in the experiments together with the following two lines *a*81 and *a*33:

*a*33 From Sinai, Egypt. Considerably later in development than the other summer-annual lines. The shape of the leaf is triangular and profusely indented. The flowers are somewhat larger than those of *L. amplexicaule* in general.

*a*81 From Bozdag near Istanbul, Turkey. Resembling *a*33, but not so tall; very hairy and even later in development. Does not flower every year.

*a*83 From Ankara, Turkey. Palmately-lobed leaves. Grows generally into bush-shaped sterile plants, but may flower very sparingly in favourable cases.

The Herbarium, Royal Botanic Gardens, Kew, after kindly examining the dried material of these three lines as well as material belonging to other species, state that they could not place them taxonomically but consider them more related within themselves than with *L. amplexicaule*, to which species they are not considered to belong. The work of classification is made more difficult by the fact that these lines were cultivated in greenhouses under conditions abnormal to the species in question. Particularly in regard to *a*33 and *a*83, this strikes me as perhaps a little too categorical; from a morphological point of view it appears to me that they might be accepted as extreme variants of *L. amplexicaule*. Dried material of these and other lines will be delivered to the Herbarium, Royal Botanic Gardens, Kew. A plant of each of *a*13 and *a*33 are reproduced in Fig. 1; *a*13 typifies the appearance of pure *L. amplexicaule*.

Information obtained from several floras covering South-Eastern Europe and Western Asia (BOISSIER, 1879; VELENOVSKY, 1891; POST, 1933; HAYEK and MARKGRAF, 1931, and others) regarding various varieties and subspecies of *L. amplexicaule*, and still more investigations of herbarium material from the territories in question, indicate an abundance of varieties within *L. amplexicaule* and its closely related spec-

ies, which is obviously larger there than in other parts of the distribution area for this species and group of species. The types mentioned above which differ more or less from the typical *L. amplexicaule*, are presumably odd examples of this variation. The mere existence of what

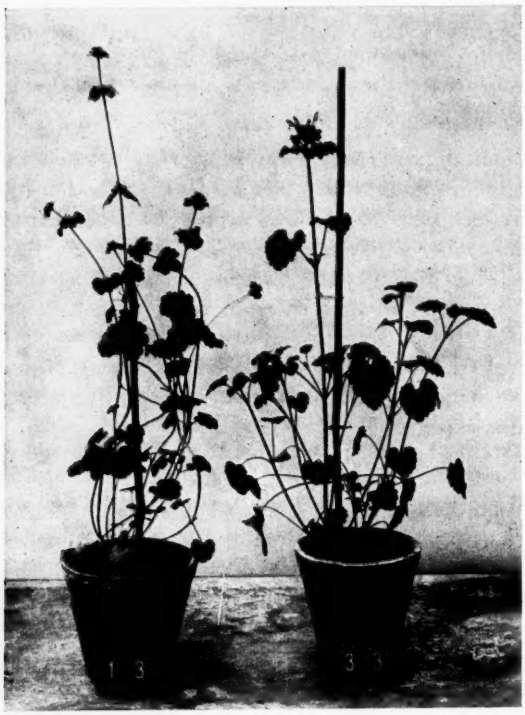


Fig. 1. *Lamium amplexicaule*. To the left: *a*13; to the right: *a*33. (*Cf.* the text.)

seems to be a variety centre for *L. amplexicaule* and its closely related forms is of interest for the present investigation (see below).

As can be seen from the survey of the lines used, their morphological variation is considerable, and this variation is characteristic of the species; this is shown by the material cultivated experimentally and by that used in the experiments, as well as by investigations of herbarium material from Europe.

All the *L. amplexicaule* lines were checked and found to have $2n=18$; this was also the case with *a*33, *a*81 and *a*83.

The fertility of the pure lines was originally examined, partly for the purpose of a comparison with that of newly produced autotetraploids and partly for comparison with the fertility of different intraspecific hybrid combinations, including a few of the pure lines listed above. It was found that while the pure lines always had perfectly good pollen, this latter was inferior in the hybrids; at the same time, the seed-setting often showed completely significant deteriorations in comparison to the parent lines. Renewed tests with more lines confirmed the first observations and showed that, as a rule, partial sterility appears to characterize interlinear hybrids of *L. amplexicaule*; also that heterosis was often a feature of the hybrids, and that meiotic disturbances (inversion bridges) were found to occur.

These conditions recall those which MÜNTZING (1929, 1930, 1932 a, 1938, 1945 a) established with respect to *Galeopsis Tetrahit*, a comparatively near relative of *Lamium*. *L. amplexicaule* being a diploid in contrast to *G. Tetrahit*, there was reason to believe that the sterility in hybrids of the former could easily become even stronger than in those of the latter species, and possibly the degree of sterility could show a positive connection with the degree of cytological disturbances, which MÜNTZING (1938) failed to find in *Galeopsis*. A possible existence of clear structural differences between lines of *L. amplexicaule* would be of great interest for parallel investigations into species synthesis, and of genomes distinguishable, within *Lamium*.

This, together with other problems to which we will revert later on, led to experiments in 1948—1949 for the purpose of obtaining a clearer conception of the intraspecific sterility of *L. amplexicaule* and questions related thereto, by means of comprehensive crossings between 10 different lines of this species, including *a*33, as well as by crossing some new lines with one or two previously tested. Failure of seed-setting in a few of the cross-combinations detracted somewhat from the value of the test; it also proved more difficult than expected to obtain satisfactory fixations of the meiosis. In spite of these difficulties the experiment gave a good picture of the relationships between the *L. amplexicaule* lines.

A. FERTILITY.

a. Pollen fertility.

The pollen fertility was analysed in all *L. amplexicaule* lines and closely related varieties, excluding *a*83 and *a*88, used in the crossing-

TABLE 2. *Pollen fertility in interlinear F_1 hybrids of L. amplexicaule, and in F_1 hybrids between L. amplexicaule and a33, a81 and a83.*

Year	Cross combinations	Percentage of good pollen grains 10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	*n*	$\bar{x}$
44—1, 49	*a*8 × *a*9					1	1	6	5	6	6	—	1								26	44,4
44—1	*a*8 × *a*10														2	2	4				8	78,6
49	*a*8 × *a*12																1	3	7	2	13	91,1
46, 49	*a*8 × *a*13													2	2	9	11	1			25	78,9
49	*a*8 × *a*32												1	1	1	2	1				6	72,9
49	*a*8 × *a*78																		1	11	12	97,1
49	*a*8 × *a*86																			7	7	98,0
49	*a*8 × *a*88								1	3	4	4									12	51,8
49	*a*9 × *a*10																		1	1	2	94,3
49	*a*9 × *a*12										2	—	—	1	—	1	1				5	67,1
49	*a*9 × *a*13																			9	9	98,1
49	*a*9 × *a*32																		2	2	4	94,2
49	*a*9 × *a*78													2	2	6	1				11	75,9
49	*a*9 × *a*86											1	1	—	3	6					11	72,4
49	*a*9 × *a*88					1	1														2	31,4
49	*a*10 × *a*12											1	—	1							2	62,9
49	*a*10 × *a*13																		1	6	7	97,9
49	*a*10 × *a*32																				—	—
49	*a*10 × *a*78													1	1	3	1	1			7	77,8
49	*a*10 × *a*86													1	—	—	1				2	75,6
49	*a*10 × *a*88																				—	—
49	*a*12 × *a*13												3	2	—	3					8	66,3
49	*a*12 × *a*32											2	1	1	2	—	2				8	69,5
49	*a*12 × *a*78																1	2	7	2	12	91,4

| | | |

tests. The tests cover up to seven generations (*a*8), and from 2 (*a*106) to 71 plants of the same line were examined.

The lowest average ascertained for line and generation is 97,9 % (*a*13—46). For the rest, the average values exceeded 98 %. Out of a total of 266 plants examined within 14 lines, *a*33 and *a*81 included, only 2 (leaving out of account the plants of *a*9—44—2 mentioned previously) had a pollen fertility ranging between 90 % and 95 %; all the others had > 95% of good pollen grains. The averages for the different lines, covering all the generations investigated, varied between 98,4 % and 99,9 %.

Thus, the pollen fertility in pure lines of *L. amplexicaule* is exceptionally high and even. According to verbal information from MÜNTZING, his figure of 95 % for this species was founded on estimations (MÜNTZING, 1939, p. 336).

Data regarding pollen fertility of the line hybrids are set out in Table 2. In cross-combinations studied during more than one generation, all the plants are grouped together, as the averages for the different generations are in good agreement. Thus, the averages for *a*8×*a*9 were 41,0 and 47,8, respectively (14 and 12 plants), for *a*8×*a*13 80,0 (12) and 77,9 (13), respectively, and for *a*8×*a*33 48,2 (7) and 42,3 (12), respectively.

No distinction has been made between reciprocal hybrids, since the values pertaining to these were always found to be in good harmony with each other. In 21 combinations, those involving *a*33, *a*81 and *a*83 included, reciprocal hybrids were studied. Pollen fertility has been studied in altogether 47 combinations; in 2 other combinations the chasmogamic flowering was too weak to allow pollen to be gathered in time.

A glance at Table 2 and its collection of hybrids within pure *L. amplexicaule* shows at once that, in by far the greater number of the hybrids, the pollen fertility is considerably lower than in the pure lines. Only 3 combinations have averages of 98 % or more, thereby approximately reaching the prevailing level of the pure lines. 2 combinations have an average of 97 %; of these, one may be as fertile as its parent lines, but it is also very possible that even this slight decrease in pollen fertility is genotypically conditioned in both of them. This cannot be appropriately examined statistically in the case of *a*10×*a*13, as pollen samples of only 2 plants of the one parent line, *a*10, could be studied in this generation. In the case of *a*8×*a*78, on the other hand, where a pollen fertility of 97,1 % might not superficially be taken to in-

dicate a real deterioration, the pollen fertility proves to be significantly inferior to that of the parent lines during 1949. The averages are:

*a*8	99,29 ± 0,232	n=14
*a*78	99,69 ± 0,118	n=14
*a*8×*a*78	97,13 ± 0,404	n=12

The standard error of the arithmetical mean of the averages of the parent lines was obtained according to the formula $\frac{\sqrt{m_1^2 + m_2^2}}{2}$, where m_1 and m_2 represent the standard errors of the averages of the parent lines (JOHANNSEN, 1926). The t-value of the difference between the mean value of *a*8×*a*78 and the arithmetical mean of the parent lines is 5,57 ($P < 0{,}001$; 37 df).

Thus, it can confidently be taken for granted that already the slight increase in the number of poor pollen grains in *a*8×*a*78 is genetically conditioned. Consequently, this combination shows that real partial sterility can border closely upon full fertility, and that a careful method must be employed in deciding pollen fertility, if approximately correct results are to be obtained.

On the other hand, *a*13×*a*32 is an example of equally high fertility in the hybrids as in the parent lines. The averages are:

*a*13	99,53 ± 0,228	n=13
*a*32	98,44 ± 0,241	n=12
*a*13×*a*32	98,64 ± 0,362	n= 8

The t-value for the difference between the arithmetical mean of the averages of the pure lines and that of the hybrids is 0,9.

Lastly, as a border-line case between the two examples given above, the combination *a*9×*a*13 may be quoted. The values are here:

*a*9	99,26 ± 0,197	n=13
*a*13	99,53 ± 0,228	n=13
*a*9×*a*13	98,07 ± 0,406	n= 9

The t-value, analogously with the above mentioned, is 3,1 ($0{,}01 > P > 0{,}001$). Thus, it is probable that already this very slight decrease in the pollen fertility by little more than 1 %, is a real one.

With increased sterility, the degree of fertility reduction varies greatly between the combinations; also, the variability within the hybrid combinations is increased. The good agreement between the averages from different years in corresponding combinations, referred to above, which caused the summation of values from different generations of 3 combinations in Table 2, shows, however, that every combination is

TABLE 3. *The distribution of mean values of pollen fertility in interlinear F_1 hybrids of L. amplexicaule and in hybrids between L. amplexicaule and a33, a81 and a83.*

Kind of hybrids	Mean values of pollen fertility																*n*
	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
Hybrids within *L. amplexicaule* ..			1	1	1	—	1	1	4	3	2	8	2	1	4	6	35
L. amplexicaule × *a33*, *a81* and *a83*	1	3	1	3	—	2	1	—	—	—	—	—	—	—	1		12

distinguished by a certain fixed degree of sterility. This is also the case in *Galeopsis Tetrahit* (MÜNTZING, 1938).

The distribution of the mean values for pollen fertility is shown in Table 3. The grouping applied in Table 2 between cross-combinations within pure *L. amplexicaule* and those between *L. amplexicaule* and systematically somewhat diverging varieties, is retained. The upper line of this table shows, perhaps more clearly than Table 2, that the variation in pollen fertility between the hybrid combinations is very broad and fairly evenly distributed. A comparison with a similar arrangement of hybrid values in *Galeopsis Tetrahit* (MÜNTZING, 1938, p. 122) shows no mean value below 50 % for this species, while three combinations in *L. amplexicaule* have a pollen fertility considerably lower than 50 %. This difference was theoretically to be expected and can be explained by the fact that the former species is tetraploid, whereas the latter is diploid. We shall return to this later.

Judging from the two tables compared with each other, partial sterility is far more general in *L. amplexicaule* than in *G. Tetrahit*, even in view of the fact that the border-line between fertility and partial sterility in the former was drawn at 98 % of good pollen, but in the latter at 90 % (MÜNTZING, 1938). Approximately 6(—9) % of the combinations examined are fully fertile in *L. amplexicaule* against 45 % in *G. Tetrahit*. This wide difference may partly be attributed to the actual experimental material of the respective species, or to the fact that the pollen fertility in the pure lines of *G. Tetrahit* is not at all so high and even as that of *L. amplexicaule* (*cf.* Table 1, MÜNTZING, 1938), which with a less scrupulous method, *i. e.* estimation, and in cases of weak sterility, may render the differences obscure, Mainly, however, it is probably caused, as in the case of the lowest mean values, by the difference

in chromosome numbers. The problems connected with this will be dealt with later.

As all the possible cross-combinations could not be produced, nor pollen samples be taken from all those cultivated, it is not feasible to investigate more closely the possible existence and the degree of differences between the lines with regard to the frequency and the degree of partial sterility in hybrids with the other lines. As in the case of *G. Tetrahit*, such differences probably occur even in *L. amplexicaule*. *a*88, which unfortunately is only included in 4 out of 8 possible combinations, contributes, for example, towards conspicuously strong sterility in all of them. The combinations with *a*78, on the other hand, all of them show comparatively high values, excepting that with *a*88. The 8 combinations with *a*9 further show a clearly greater distribution towards stronger sterility than those with *a*78. This may be illustrated by the following table:

	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
*a*9×8 lines	1			1					1	1	1			1	2
*a*78×8 »								1			3	1		1	2
*a*88×4 »	1	1				1		1							

As only 4 of the 9 lines used were tested with the complete number of possible combinations, the exemplification above is probably not as good as could have been the case, had a more complete material been available.

The absence of values for 5 combinations also renders impossible direct tests in order to ascertain whether the lines used can be divided into groups or types, within which the F_1 hybrids between the lines included are entirely fertile, while the hybrids between the group lines and each one of the others included in the test show the same degree of fertility; thus, the factors producing sterility would be the same for all lines within one group. MÜNTZING showed with 12 tested lines of *G. Tetrahit* that these must be divided into 10 such groups, by joining 3 in one group, while each of the other lines represented a type of its own. There was the possibility, however, that if further lines had been tested, the 3 lines which appeared similar in the test might have been found to be dissimilar also.

It is indirectly possible in *L. amplexicaule*, too, to test the number of types for sterility causes covered by the lines used. Testing is facilitated by the very small number of fully fertile combinations. Generously estimated, these are *a*8×*a*86, *a*9×*a*13, *a*10×*a*13 and *a*13×*a*32. *a*8 and

*a*86 cannot belong to the same type, as their respective hybrids with *a*9 show highly different fertility. *a*9 and *a*13 are in the same way dissimilar in their hybrids with *a*8, and *a*13 and *a*32 differ in their hybrids with *a*86. *a*10 and *a*13 are not so clearly defined as the others. The lines in combinations with which *a*10 and *a*13 are somewhat different, are *a*9, *a*86 and *a*33, which latter for this purpose at least is undoubtedly equal to the other lines studied.

The difference between the mean values of *a*10×*a*86, which is unfortunately based on 2 plant-values only, and *a*13×*a*86 is rather great but not significant. On the other hand, the t-value is $2{,}_{54}$ ($0{,}_{05} > P > 0{,}_{02}$) for the difference between the mean values of *a*9×*a*10 and *a*9×*a*13, and the t-value for the difference between *a*10×*a*33 and *a*13×*a*33, is $2{,}_{36}$ ($0{,}_{05} > P > 0{,}_{02}$). The fact that two t-values of rather high order were obtained from 6 available comparisons, would seem to indicate that available data contradict rather than substantiate a type resemblance between *a*10 and *a*13. There is the additional point that the seed-setting of *a*10×*a*13 is fairly significantly inferior to that of its parent lines (see below).

Thus, among the pollen-fertile combinations there was only one, *a*10×*a*13, in which the respective lines could possibly, albeit doubtfully, belong to the same type or group. It now remains to be examined whether the lines in any of those cross-combinations, whose pollen fertility could not be investigated, or which were unobtainable, could in their turn form a group. These combinations are *a*10×*a*32, *a*10×*a*88, *a*12×*a*88, *a*32×*a*88 and *a*86×*a*88.

With reference to the fertility of the F_1 hybrids, the lines in all the 4 combinations with *a*88 reacted differently from *a*88 when hybridized with, for example, *a*8; thus, none of them can belong to the same group as *a*88. On the other hand, the fertility is identical in all F_1 hybrids with *a*10 and *a*32, respectively, including hybrids with *a*33. It is possible, then, that *a*10 and *a*32 belong to the same type with regard to factors producing sterility. As pointed out above, none of them were tested in combination with *a*88; therefore, their possible relationship was not so thoroughly examined as that of several other lines.

Summing up, it may be said that most of the lines studied represented constitutional types of their own with regard to sterility-producing factors, although perhaps 2 of the lines, *a*10 and *a*32, might be of the same type; possibly — although this is very doubtful — *a*10 and *a*13 may also form a group. It is then within the bounds of possibility that *a*10, *a*13 and *a*32 form a common group. It must, however,

always be borne in mind that if further lines are included in the experiments, constitutional differences might very possibly appear between lines which at first seemed to belong to the same type. MÜNTZING (1938) thus showed with an analogous material within *G. Tetrahit* that hybrids between 2 lines need not be fertile even if both produce fertile hybrids with a third line. The characteristics found in *L. amplexicaule* seem to agree so much in nature with those of *G. Tetrahit* — nothing has appeared to contradict this — that one may expect to find the essential characteristics of *Galeopsis* also in *Lamium*.

Up to now we have dealt with the upper sections of Tables 2 and 3. The bottom section gives the values of F_1 hybrids between pure *L. amplexicaule* and the lines *a*33, *a*81 and *a*83 morphologically separated from the former. It will be seen at once from Table 2 that *a*83 must reasonably be classed as belonging to *L. amplexicaule*, even if its appearance does not correspond fully with that of the pure species, since its hybrids with *a*8 have perfectly good pollen. For the present at least, this line should therefore be regarded as an ecotype of *L. amplexicaule*, in the meaning applied to this term by CLAUSEN, KECK and HIESEY (1945).

*a*33 diverges strikingly from the other comprehensively tested lines by reason of the low pollen fertility of all its F_1 hybrids with lines from pure *L. amplexicaule*. Presumably *a*81 might resemble *a*33 if more extensive investigations were made, but hitherto the sterility of its F_1 hybrids is not greater than, for instance, that of *a*88 in F_1 hybrids with *a*9 and *a*13, respectively. Scanty and sporadic flowering presented obstacles to the use of *a*81 in these experiments; *i. a.*, it would have been of interest to study its hybrids with *a*33. For the present, *a*81 must be left out of consideration.

In 5 of the combinations with *a*33, reciprocal hybrids were studied, and their degree of sterility always corresponded well. Thus, the sterility is not cytoplasmatically conditioned. It is then to be assumed that the throughout pronounced sterility of the hybrids between *a*33 and lines of pure *L. amplexicaule* is caused by the existence and co-operation of either more or stronger sterility-producing factors than are generally found in hybrids within *L. amplexicaule*, or by both these factors. The cytological studies point to both these possibilities (*cf.* below). The fact that clear differences, which in several cases must be quite significant, exist between the different combinations with *a*33, seems to imply that the same sterility-producing factors which are active within pure

L. amplexicaule make their influence felt between *L. amplexicaule* and *a*33, too.

The pronounced sterility throughout the hybrids with *a*33 in combination with the different morphological and physiological features, which distinguish *a*33 from the typical *L. amplexicaule,* show that this line cannot belong in a restricted sense to the species mentioned. *a*33 should rather be regarded as a representative of a separate ecospecies; investigations of offspring from *a*33×*L. amplexicaule* would, however, have been desirable as a basis for a more definite opinion. We will revert to this question, as well as to that of terminological characterization of the pure lines within the pure species, when discussing the intraspecific sterility found within *L. amplexicaule.*

b. Seed fertility.

The seed fertility in the diploid *L. amplexicaule* has been touched upon in an earlier paper (BERNSTRÖM, 1950 b), in which it was shown that the seed-setting of the chasmogamic flowers of *a*8 is inferior to that of the cleistogamic, while no such difference exists in *a*32. It was further shown that self-pollination in approximately 10 % of the chasmogamic flowers of both lines fails. Self-pollination in the cleistogamic flowers, on the other hand, functions most satisfactorily. As the chasmogamic flowers largely or entirely develop in the summer, simultaneously with the cleistogamic, while the latter universally prevail in the autumn, the result is that the seed-setting of the summer generations of *a*8 in particular, which also has a more profuse chasmogamic flowering than *a*32 (see above), is inferior to that of the plants grown in the autumn.

The seed-setting in the pure lines and the F_1 hybrids between the lines is given in Tables 4 and 5, of which Table 4 in particular illustrates what has been said above. In calculating the average value for *a*9—44—2, the semisterile plants referred to on page 170 were omitted.

From the tables it will be seen that the seed-setting of pure lines best attains the same high percentage as the pollen fertility always shows. The values for the majority of lines during 1949, for example, are only slightly below those which, as a rule, characterize the pollen fertility. Thus, under favourable conditions the seed-setting is extremely good.

The interlinear differences in seed-setting between the pure lines, other than *a*8 and *a*32, respectively, in the summer generations in Table 4, are probably also attributable to the chasmogamic flowering

TABLE 4. *Seed-setting in pure lines of L. amplexicaule and their F_1 hybrids in 1944—1946. (Cf. the text.)*

Generation	44—1			44—2			45—1			45—2			46		
Lines and hybrids	$\bar{x}$	rel. value	*n*	$\bar{x}$	rel. value	*n*	$\bar{x}$	rel. value	*n*	$\bar{x}$	rel. value	*n*	$\bar{x}$	rel. value	*n*
							Pure lines								
*a*8	80,3 ± 2,20		14	98,3 ± 0,45		12	72,9		14	90,1 ± 1,41		11	84,4 ± 3,80		5
*a*9	92,9 ± 1,10		21	96,9 ± 1,01		8	89,8		14	93,5 ± 1,17		12			
*a*10	97,7 ± 0,91		14	98,0 ± 0,74		9	95,0		14	94,7 ± 0,84		12			
*a*12							77,9		7	91,6 ± 1,27		11			
*a*13							(91,0		2)	94,6 ± 0,70		12	92,5 ± 0,81		8
*a*32				94,5 ± 1,00		13	84,1		14	90,7 ± 2,01		12	92,3 ± 1,59		8
*a*33							90,0		7						
							F_1 hybrids								
*a*8 × *a*9	52,6 ± 2,34	61	14	68,3 ± 2,11	70	8									
*a*8 × *a*10	86,0 ± 0,74	97	10	90,4 ± 0,87	92	7									
*a*8 × *a*12										84,8 ± 0,85	93	9			
*a*8 × *a*13													88,6 ± 0,92	100	13
*a*8 × *a*32				84,5 ± 1,13	88	14									
*a*8 × *a*33				37,4 ± 1,60	—	13									
*a*9 × *a*10	97,2 ± 0,66	102	7	91,1 ± 1,60	93	7									
*a*9 × *a*12										68,1 ± 2,26	74	8			
*a*9 × *a*13										89,8 ± 0,84	95	8			
*a*9 × *a*32										85,3 ± 1,43	93	12			
*a*12 × *a*13										85,7 ± 1,75	92	10			

TABLE 5. *Seed-setting in pure lines of L. amplexicaule and their*

(The number of

	*a*8	*a*9	*a*10	*a*12	*a*13	*a*32	*a*33	*a*78
*a*8	88,9 ± 1,52 (14)	69,6 ± 1,80 (12)	—	80,7 ± 1,95 (13)	91,4 ± 1,22 (13)	87,8 ± 1,32 (7)	39,5 ± 1,88 (12)	91,9 ± 1,69 (13)
*a*9		98,1 ± 0,42 (14)	99,0 ± 0,62 (7)	62,8 ± 2,17 (15)	92,7 ± 0,70 (14)	90,9 ± 1,89 (8)	30,1 ± 2,13 (15)	95,3 ± 0,57 (14)
*a*10			98,5 ± 0,50 (19)	78,2 ± 1,69 (10)	94,7 ± 1,04 (9)	96,4 ± 0,76 (13)	28,9 ± 1,76 (9)	94,4 ± 0,75 (9)
*a*12				94,5 ± 1,12 (14)	79,9 ± 1,19 (14)	53,2 ± 1,32 (11)	26,7 ± 2,03 (14)	67,2 ± 1,89 (14)
*a*13					98,2 ± 0,46 (13)	96,7 ± 0,87 (9)	30,4 ± 3,55 (10)	83,1 ± 1,41 (14)
*a*32						97,2 ± 0,51 (13)	25,6 ± 1,43 (10)	88,5 ± 1,06 (10)
*a*33							79,9 ± 2,16 (12)	38,0 ± 1,58 (12)
*a*78								98,9 ± 0,40 (14)
*a*86								
*a*88								

(Pure lines):

and its various degrees in the respective lines. The mean values of the autumn generations (years 1944 and 1945) show a considerable degree of uniformity, which must be ascribed to the then almost purely cleistogamic flowering. The differences between generations of the corresponding category are, presumably, due to seasonal variations.

The flowering during 1946 and 1949 occurred, with regard to the time of year, approximately between the summer and autumn generations in 1944 and 1945. This is apparent by the amount of difference between the mean value for *a*8 and that for the lines with the highest fertility during the summer (*e. g.*, *a*10); the greater this difference is in a generation, the richer has the chasmogamic flowering generally been. Direct observations of the extent of the chasmogamic flowering (it was

F_1 hybrids in 1949, including those with a33, a81 and a83.
individuals in brackets.)

a78	a86	a88	a72	a81	a83	a106	a108	a109
,0 ± 1,89 (13)	91,3 ± 1,93 (8)	72,4 ± 1,75 (14)	90,7 ± 0,64 (10)	54,7 ± 1,81 (8)	90,2 ± 0,88 (10)	88,9 ± 1,02 (8)	81,4 ± 1,16 (10)	68,3 ± 2,25 (11)
,3 ± 0,57 (14)	66,4 ± 1,32 (12)	50,4 ± 4,47 (4)						55,3 ± 2,55
,4 ± 0,75 (9)	84,2 ± 1,79 (10)	56,7 ± 3,19 (8)						
,2 ± 1,89 (14)	80,1 ± 1,93 (13)	—						
,1 ± 1,41 (14)	76,9 ± 1,87 (14)	47,9 ± 1,87 (10)						
,5 ± 1,96 (10)	71,6 ± 1,57 (10)	—						
,0 ± 1,58 (12)	21,3 ± 2,02 (13)	51,9 ± 1,92 (9)						
± 0,40 (14)	73,6 ± 2,49 (13)	67,0 ± 1,41 (10)						
	98,5 ± 0,36 (12)	—		36,6 ± 1,13 (5)				
		97,0 ± 0.87 (12)						
			99,0 ± 0,34 (7)	—	97,7 ± 2,00 (4)	97,1 ± 1,03 (5)	93,4 ± 1,15 (6)	96,1 ± 0,84 (7)

unfortunately impossible to register the latter carefully to any great extent) well support the values for the seed-setting.

The low seed fertility of *a*33 in 1949 (Table 5) was probably not occasioned by rich chasmogamic flowering, because the seed-setting was clearly better during the summer of 1945, when the mean value for *a*33 was 90,0, while, at the same time, the chasmogamic flowering was far better than in 1949, but must rather be assumed to be a consequence of unsuitability of the northern climatic conditions for this line, which has its origin in Egypt.

Like the pollen, the seed-setting in the hybrids is generally characterized by deterioration in comparison to that of the parent lines. The degree of reduction in seed fertility has, obviously, also a certain

definite order of magnitude for each hybrid combination. This is evident from several facts, among them from comparisons between the seed-setting of the same combinations in different generations. Thus, a comparison of the mean values for seed-setting in the 11 combinations which were studied 2 or 3 times (Tables 4 and 5) will show, in the case of at least 10 of them, a fair degree of correspondence between the generations. The generally minor divergences between generations which characterize a few of the hybrid combinations, are probably caused by different degrees of chasmogamic flowering, and by seasonal variations, in the same way as is the case with the pure lines. The differences between the mean values of $a8 \times a9$ in 1944—1, 1944—2 and 1949, respectively, are probably a consequence of chasmogamic flowering; *cf.* the seed-setting of *a*8 and *a*9 in the same generations.

$a9 \times a10$ seems to be the only exception to the rule that there is a certain, definite degree of seed-setting for each hybrid combination. $a9 \times a10$ had a seed fertility during 1944—1 of 97 %, and during 1949 of 99 %, but only 91 % during 1944—2 when the seed-setting of the parents was between 97 % and 98 %. The 1944—2 deviation from the mean values of the other 2 generations may appear illegitimately large, as this combination seems to be generally a fully fertile one. The pollen samples of 1949, unfortunately only 2 in number, indicate however that the fertility of $a9 \times a10$ is not quite perfect; the percentage of good pollen grains was approximately 93 and 96, respectively, which, in view of the evenness of the pollen fertility of the pure lines is a striking reduction. The uneven seed-setting values of $a9 \times a10$ may in that way signify unusually uneven manifestations of a weak fertility reduction.

Another indication that each hybrid combination is characterized by a certain definite degree of reduction in seed fertility, is to be found in the size and evenness of the standard errors shown in Table 5. Had not the degree of seed-setting been definite for each combination, the variation within the combinations could reasonably be expected to have been far greater than it actually was. The seed-setting of reciprocal hybrids proved independent of the direction of the crossing throughout. For that reason, as in the case of the pollen fertility, only the common value of every combination is quoted.

Both pollen fertility and seed fertility thus having a characteristic size in each hybrid combination, it is of interest to determine the degree of correlation between them. Combinations with *a*33 and *a*81 have been included in the calculation. Based on 46 hybrid combinations, cultivated

in 1949, the correlation coefficient is +0,83 (t=9,80); thus, the correlation between pollen and seed fertility is highly significant in this material.

It should be pointed out in this connection that within the single hybrid combinations no constant advantage over the other of either kind of measure of the fertility is at hand; in other words, the pollen fertility is as often higher than the seed fertility as *vice versa*. This shows, together with the correlation coefficient obtained, that the conception of the percentage of good pollen as an indirect measure of the real fertility, which is the seed-setting, is upon the whole correct for this material, and that the term pollen fertility for the percentage of morphologically good pollen is well-grounded. It is not certain that this is always the case, and sometimes the idea of the use of »pollen fertility» as a measure of the real fertility is evidently rather loosely based and not proved experimentally. In many cases, especially in allogamous species and polyploids, the correlation between the percentage of good pollen and the degree of seed-setting has also appeared to be slight or non-existent; *cf.*, *e. g.*, DERMEN (1940), BAYLISS (1936; quoted from LAMM, 1945), MÜNTZING (1946).

Though the cases of the pollen fertility being superior to the seed fertility were about as frequent as the opposite, it has been of interest to establish whether there were any differences between the lines used in the crossing experiment of 1949, with reference to ability of producing preponderance for one or the other measure of the fertility. Therefore, the difference between seed fertility and pollen fertility was computed for every combination, and the averages of the differences within the respective lines in question estimated. They varied from +9,0 for *a*88 to —9,7 for *a*33. *a*33 was the only line which had the same sign (+ or —) in all its hybrid combinations, the seed fertility of these invariably being lower than the pollen fertility. On the other hand, it should be noted that the opposite extreme is *a*88 which, next to *a*33, is distinguished by low fertility of its hybrid combinations.

By analysis of variance of the material, *a*33 was *a priori* compared with the other lines on account of its pronounced partial sterility barrier against the lines of pure *L. amplexicaule* and its diverging taxonomic characteristics. It was found that *a*33 differs rather significantly from them, while on the other hand no significant differences exist between the lines of pure *L. amplexicaule*.

This shows a tendency on the part of *a*33 to present, in the relation between the two measures of the fertility, the same characteristics as

	Df	Sum of squares	Mean square
between *a*33 and other lines	1	960,13	960,13
» other lines	8	1777,09	222,14
within »	68	9995,08	146,99
total	77	12732,30	

$\frac{960,13}{146,99} = 6,53$ $(0,05 > P > 0,01)$ $\frac{222,14}{146,99} = 1,51$ (P *ca.* 0,2)

species hybrids, polyploids, and other more or less pronounced sterile forms of *Lamium*, and probably of most other genera, too (BERNSTRÖM, 1944, and unpubl.). In species hybrids, and others, the inferiority of the seed-setting in relation to the pollen fertility, is at least partly a consequence of difficulties with regard to self-fertilization; it has not been investigated whether similar difficulties exist as far as the intraspecific hybrids with *a*33 are concerned. It is possible that the poor seed fertility of *a*33 in 1949, which has been touched upon above, may recur in hybrids with that line too; but it is surely more reasonable to suppose that an important cause of the throughout inferior seed-setting in hybrids with *a*33, as related to the pollen fertility, is to be found in genetically conditioned disturbances of the complicated and delicate process of pollination, with subsequent fertilization and embryo development (*e. g.*, zygotic lethality). Disturbances of a similar nature undoubtedly occur to an even higher degree in species hybrids where, in combination with pollination-mechanical difficulties, they contribute towards causing inferior seed-setting.

The difference in behaviour between hybrids of *a*33 and those of the other lines is further illustrated by Table 6. In this table the differences between seed fertility and pollen fertility have been correlated with the pollen fertility of all the hybrid combinations studied in 1949. Combinations including *a*33 are in italics, and those with *a*81 in fat types. Viewed as a whole, it only very faintly indicates a negative connection between a change-over from a subordinate position to predominance of the pollen fertility over the seed fertility on one hand, and a general increase in the pollen fertility on the other. The correlation coefficient is —0,19. If, however, the combinations with *a*33 and *a*81 are separated from the others — and this may be justified on the same grounds as the exclusion of *a*33 in the analysis of variance above — the correlation of the remaining lines becomes highly significant, the correlation coefficient being then — 0,66 ($t = 5,05$). The significant change in the relation between pollen fertility and seed fertility, which has been pointed to above in the analysis of variance, reserves for *a*33 and

TABLE 6. *Correlation between pollen fertility and difference between seed fertility—pollen fertility in interlinear F_1 hybrids of L. amplexicaule in 1949.*

(Hybrids with *a*33 are in italics, those with *a*81 in fat types.)

Pollen fertility	Difference between seed fertility–pollen fertility														*n*
	−33	−28	−23	−18	−13	−8	−3	+2	+7	+12	+17	+22	+27	+32	
28							*1*	*2*	**1**			1			1 + 4
36					*1*	*1*		*1*		1					1 + 3
44		*1*			*1*					**1**		2			2 + 3
52							*1*							1	1 + 1
60							1		2		2				5
68					1		1		1	2	1	1			7
76							1		1	1	2				5
84			1			1	1	1							4
92				1		1	5	1	1						9
100															
		0 + 1	1	1	1 + 2	2 + 1	9 + 2	2 + 3	5 + 1	4 + 1	5	4	—	1	35 + 11

a) All hybrids:
r = — 0,19
t = 1,28
b) Hybrids within pure *L. amplexicaule*:
r = — 0,66
t = 5,05 ($P < 0{,}001$)

possibly also for *a*81 a position in Table 6 which is inconsistent with the correlation characteristic for the rest of the material.

Taking pollen fertility as a gauge of the fertility, we find, consequently, that there is in interlinear hybrids within pure *L. amplexicaule* a connection between the fertility on one hand, and the relation between seed- and pollen-fertility on the other, which in the case of the more sterile hybrid combinations is, broadly speaking, the reverse of that which generally applies to partially sterile varieties of different kinds in *Lamium* (*cf.* above). In the combinations showing the most pronounced sterility, the seed-setting is, as a rule, considerably better than the pollen fertility, while at a level of $^3/_4$ fertility both fertility values become on the whole equal. In cases of slightly reduced or full fertility, the pollen fertility generally preponderates.

The cause of the conditions explained may be assumed to be the absence of more profound genetical divergencies between the lines of pure *L. amplexicaule*. Probably, the sterility is principally caused by small chromosome-structural dissimilarities (*cf.* below), and it is a well-established fact that pollen grains are more sensitive to structural chromosome alterations than the ovules (*cf.*, *e. g.*, MÜNTZING's review, 1945 b). Thus, the pollen fertility will be lower than the seed fertility at stronger haplontic sterility; with a weaker degree of sterility, on the other hand, a change the other way round is brought about as a result of flower-biological factors, in this material perhaps principally the chasmogamic flowering. A contributory cause may be found in genetically conditioned disturbances, too; however, these will then occur to a less extent than in the hybrids between *a*33 and pure *L. amplexicaule*.

c. Seed fertility in autotetraploid hybrids.

Here a circumstance must be briefly touched upon which properly pertains to another connection and will be dealt with more fully in a later paper. It is, however, also of importance to the present topic since it shows that the partial sterility in at least 4 of the cases studied cannot be genic in the meaning used by DOBZHANSKY (1941) with reference to similar cases, nor diplontic in MÜNTZING's meaning (1930). It is here a question of hybrids between autotetraploid lines of *L. amplexicaule*, originating from diploid lines, which were used in the crossing experiments described above. With a view, partly, to comparing their fertility in relation to that of the parent lines with the fertility of the corresponding diploid hybrids, the following 4 tetraploid interlinear hybrids were studied:

*a*14×*a*15,	corresponding	to	the	diploid	*a*8×*a*9
*a*14×*a*17	»	»	»	»	*a*8×*a*10
*a*14×*a*27	»	»	»	»	*a*8×*a*12
*a*14×*a*28	»	»	»	»	*a*8×*a*13

Attempts have been made to produce further combinations than those mentioned, but seed-setting is very low in tetraploid *L. amplexicaule* when cross-fertilized, and this was one of the reasons for the limited scope of the experiments.

Being between 50 % and 60 % of that of the diploids, the seed-setting of tetraploid *L. amplexicaule* is reduced, as is always the case with newly produced autotetraploids; for various reasons, only the seed-setting of the hybrids was examined. It was found that the seed fertility of no combination was below the average of that of the parent lines. On the contrary, *a*14×*a*17 was significantly superior to both parents ($P < 0{,}001$ in both comparisons), and *a*14×*a*28, too, fairly certainly had a better seed fertility than its parent lines; the comparisons covered F_1—F_3, and all generations showed retained superiority. The other 2 line combinations had approximately the same degree of seed-setting as their parent lines. Apart from F_1 of *a*14×*a*27 and F_2 of *a*14×*a*28, the experiments were conducted with autumn generations; errors caused by sterility of chasmogamic flowers can, therefore, hardly have influenced the observations.

Thus, despite the fact that 3 of the diploid combinations which correspond to the tetraploid ones investigated, in repeated experiments showed, as a rule, significant reductions in their seed fertility (see Tables 4 and 5; only *a*8×*a*10—44—1 is intermediary between the parent lines on account of the abundant chasmogamic flowering in *a*8 during the summer) while the fourth combination, *a*8×*a*13, for 2 generations was intermediary in its seed-setting, although its average pollen fertility is only 79 %, no trace of this sterility was found in the combinations as tetraploid when comparing their seed-setting with that of the pure tetraploid lines. The fertility increase which now, instead, characterizes 2 of the tetraploid combinations, does not constitute anything new; a similar effect of intraspecific hybridization has been found in many genera (see, *e. g.*, MÜNTZING, 1943; LEVAN, 1946). As regards the actual material, it is interesting to find that those factors which in *L. amplexicaule* cause a reduced fertility of hybrids in the diploid condition, are obviously not the same, or only the same as those which effect an increase of the fertility when the hybrids are tetraploid; this is evident

from the fact that only 2 of the tetraploid combinations showed an increased fertility, while all the diploid ones were partially sterile.

As regards the partial sterility eliminated by tetraploidy, it is possible to establish the fact that the relations between the genes must be the same in the diploid F_1 hybrids as in their tetraploid equivalents. If the sterility in the diploids were genic, according to the terminology of DOBZHANSKY, it could not be expected that it would be eliminated by a doubling of the chromosome number; the tetraploid hybrids should even then be characterized by a fertility inferior to that of the parent lines. It is, then, a reasonable conclusion that the interlinear sterility of the diploids dealt with above cannot be genic, nor, presumably, that of the others either.

B. CYTOLOGY.

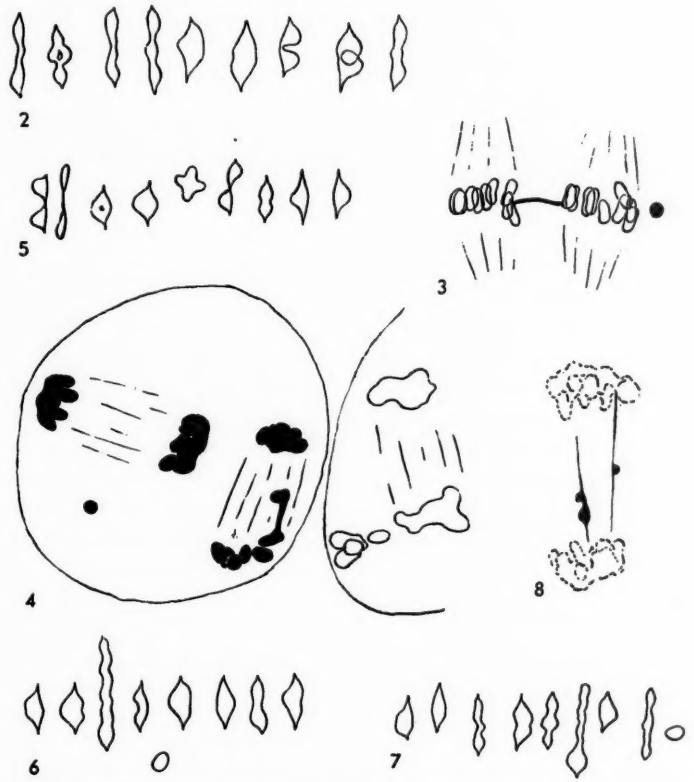
a. *L. amplexicaule.*

By counting the somatic chromosome numbers, all the lines of *L. amplexicaule* used in the crossing experiments proved to have $2n=18$. In addition, the chromosome numbers of at least a dozen other lines of this species, originating from various parts of Europe, were counted and invariably found to be $2n=18$.

Because of the amount of work necessary for obtaining usable slides, the meiosis of the pure lines of the diploid species was not given as much attention as that of the phylogenetically more important tetraploid ones, and of the various species hybrids. Preparatory studies were made of the reduction division of several pure lines of *L. amplexicaule*, but bad fixations rendered a detailed examination impossible. On the whole, however, meiosis was found to be entirely regular, and no irregularities whatever were observed. Satisfactory slides, obtained by means of fixatives containing osmic acid, were available of *a*8 and *a*12. 35 PMC's in I-M of *a*8 had exclusively 9 II; this was also the case with 30 PMC's of *a*12. Here as in the following, only cells in I-M seen from the side were counted. Neither did 38 cells in II-M nor 12 cells in II-A in *a*12 show anything remarkable.

It is to be expected *a priori* that the reduction division in a diploid, strictly self-fertilizing species like *L. amplexicaule*, with unfailingly good pollen, should be very regular. JÖRGENSEN (1927) has reproduced diakinesis and II-M in *L. amplexicaule*, but says nothing about the various stages of meiosis.

Bud fixations were made on a fairly large number of hybrid com-



Figs. 2—8. Meiosis in interlinear hybrids of *L. amplexicaule*. — Figs. 2—4, *a*8×*a*9; Fig. 2, I-M in side view (the bivalents separately drawn); Fig. 3, II-M with dicentric chromatid+acentric fragment from I-A; Fig. 4, II-A, probably a dicentric chromatid +acentric fragment in a cell showing cytomixis. — Fig. 5, *a*8×*a*12; I-M in side view (the bivalents separately drawn). The difference in size between the chromosomes in Figs. 2 and 5 is caused by different fixation methods. — Figs. 6—8, *a*33×*a*8; Figs. 6—7, I-M with 1 III+7 II+1 I (the chromosomes separately drawn); Fig. 8, I-T; two dicentric chromatids, probably with fragments. — ×3000.

binations. Only in a few cases was a combination chosen for fixation on account of unusually low fertility; as a rule, such combinations were fixed of which a sufficient material was available. Unfortunately, usable slides were only obtained from some of the combinations fixed. The results of the cytological investigation of the hybrid combinations are reproduced below. When there was nothing in the PMC's to indicate

any irregularities of the meiosis, they have been characterized as normal.

*a*8×*a*9 (1944—2).

22 cells in diakinesis. All were normal, with 9 II.

33 » » I-M. All were normal. In Fig. 2 the bivalents of a cell picked at random are drawn separately.

20 cells in I-A. 19 were normal; the 20th probably contained a broken inversion bridge, without fragment.

118 cells in II-M. All except 4 appeared normal, and in most of them the distribution of the chromosomes could be checked. Each of 3 cells had a univalent in the cytoplasm, and 1 cell had an inversion bridge between the plates in addition to a fragment (Fig. 3).

II-A was investigated in 2 anthers of the same bud. Minor irregularities were fairly common, but since cytomixis occurred in at least 1 of the anthers, no definite conclusions can be drawn from the observations. In those cells which did not seem to be directly involved in cytomictic disturbances, there were 1—4 univalents, or fragments, outside the groups; unequal anaphase groups were also observed. In 1 cell probably a real inversion bridge was found (Fig. 4). It is true that cytomictic disturbances had caused some of the chromosomes in the spindle to appear in the neighbouring cell, which admits of some doubt as to whether the bridge was a real inversion one. On the other hand, the interpretation is endorsed by the existence of what was probably a fragment, and by the typical development of the bridge between well delimited anaphase groups. As an inversion bridge, it should be the result of two crossing-overs in the inversion arm, one between the centromere and the inversion, and the other in the inversion (DARLINGTON, 1937).

*a*8×*a*12 (1945—2).

66 cells in I-M. All had 9 II except one which had 8 II+2 I. The bivalents seemed more frequently to have 2 chiasmata than 1. Because of the difficulties involved in comparative investigations of the parent lines, no investigations of the chiasma frequencies were, however, carried out neither on this nor the other species studied. The bivalents of a cell picked at random are illustrated in Fig. 5, where the bivalents have been drawn separately. The difference in size between the chromosomes in Figs. 2 and 5, among others, is due to the fact that various methods of fixation have been used.

36 cells in II-M. 2 cells had a univalent in the cytoplasm; the other cells were normal.

*a*13×*a*8 (1949).

89 cells in II-M. 87 were normal, while 2 had a univalent in the cytoplasm well outside the metaphase groups.

*a*33×*a*8 (1948).

140 cells in I-M. They were distributed as follows:

12	»	had 1 III+7 II+1 I
1	cell	» 1 III+6 II+3 I
122	cells	» 9 II
5	»	» 8 II+2 I

In Figs. 6 and 7, the chromosomes of 2 cells with 1 III+7II+1 I are illustrated (the chromosomes are drawn spread out with retained vertical positions, as is also the case wherever their positions in the equator have not been retained).

89 cells in I-A—I-T. They were distributed as follows:

65 » appeared to have normal separation.

12 » had 1 inversion bridge; 1 fragment was visible in half of them.

3 » had 2 inversion bridges; 2 fragments were probably visible in one of them (Fig. 8).

1 cell probably had 3 inversion bridges, one of which had burst (Fig. 9).

8 cells with minor irregularities of uncertain and varying nature; the majority at least were probably remnants of inversion bridges, or consisted of 1—2 fragments of different sizes in the spindle without any visible inversion bridges belonging to them. The bridges had very likely been thin or they had burst from the beginning.

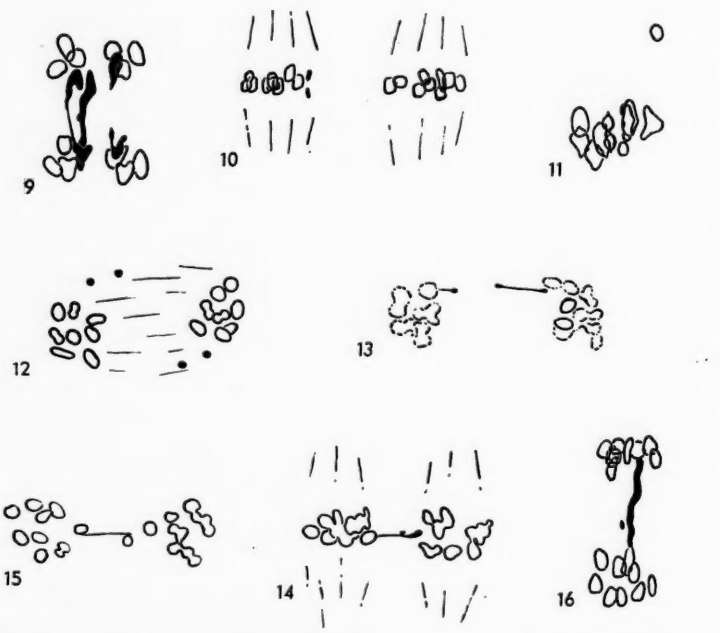
*a*8×*a*81 (1949).

119 cells in I-M. 107 were normal with 9 II, while 12 had 8 II+2 I.

8 » » I-A. All normal.

151 » » II-M. 148 cells appeared to be normal; the chromosome distribution could, however, only be checked in about $^1/_6$th of them.

2 cells had a distribution of 8—10, and the 10-group of one of



Figs. 9—16. Meiosis in interlinear hybrids of *L. amplexicaule*. — Fig. 9, *a*33×*a*8; I-A, probably three dicentric chromatids, one of which has burst. — Fig. 10, *a*8×*a*81; II-M, the appearance of one chromosome indicates that it has been stretched out between the poles during I-A as a univalent. — Figs. 11—13, *a*8×*a*109; Fig. 11, I-M with 8 II+2 I; Figs. 12—13, late I-A, each cell has two more or less stretched-out univalents in the spindle (the spindle in Fig. 12 is seen obliquely from above). — Figs. 14—15, *a*9×*a*12; II-M, dicentric chromatids from I-A, an acentric fragment visible in Fig. 14. — Fig. 16, *a*78×*a*10; I-A, with dicentric chromatid+acentric fragment. — ×3000.

the cells contained a chromosome, the stretched-out appearance of which indicated that, during the first division as a univalent, it had been stretched out between the poles and later joined one of them. The third aberrant cell had a distribution of 9—9, but one chromosome in one of the groups stood out from the others by reason of its stretched-out appearance in the same way as has just been described (Fig. 10; see also Figs. 12 and 13).

*a*8×*a*83 (1949).

23 cells in I-M. All had 9 II.

*a*88×*a*8 (1949).

14 cells in I-M. 13 had 9 II while 1 had 8 II+2 I.
28 » » II-A. 26 of these appeared to be normal, while 2 had 1 univalent each in the cytoplasm.

*a*8×*a*109 (1949).

41 cells in I-M. 39 had 9 II, while 2 had 8 II+2 I. A similar cell is illustrated in Fig. 11.
39 cells in late I-A. 36 appeared to be normal, although the distribution of the chromosomes was in most cases difficult or impossible to examine. The aberrant cells each had 2 univalents in the spindle, more or less stretched-out (Figs. 12 and 13; the cell in Fig. 12 is seen obliquely from above). The univalents in Fig. 12 may give the impression of being divided, but probably they are not. It seems more likely that their halves are attached by a thin thread as in Fig. 13. Univalents, which are undisputably divided at I-A, have in fact not been observed in *Lamium*.

*a*9×*a*12 (1949).

34 cells in I-M. 32 had 9 II, and 2 had 8 II+2 I.
16 » » II-M. 10 appeared to be normal, but 6 had an inversion bridge between the metaphase groups. 1 fragment was visible in 2 of the cells. Figs. 14 and 15 represent 2 cells, in one case with, in the other without visible fragment.

*a*86×*a*9 (1949).

8 cells in I-M. All with 9 II.
22 » » II M. 21 appeared to be normal, although bad fixations in most cases prevented examination of the distributions. 1 cell had a stretched-out univalent between the groups.

*a*78×*a*10 (1949).

138 cells in I-M. All cells except 7 had 9 II; the others had 8 II + 2 I.
21 cells in I-A. 2 of these had an inversion bridge+fragment. One of them is represented in Fig. 16.

*a*12×*a*32 (1949).

3 cells in II-M. 1 had a univalent in the cytoplasm; the others appeared to be normal.

12 cells in II-A. 4 appeared to be normal. 3, or possibly 4, of the other cells each had an inversion bridge which was broken. 2 of them had arisen in I-A, and the other in II-A. Of the other aberrant cells, 3 had a univalent in the cytoplasm, and 1 had two ditto.

*a*86×*a*33 (1949).

11 cells in I-M. 10 had 9 II, while 1 had 8 II+2 I. In a damaged cell was met with what very clearly appeared to be a V-shaped trivalent, and in another damaged cell there seemed to be a straight trivalent. It would seem, therefore, that this hybrid combination is distinguished by a translocation.

*a*86×*a*81 (1949).

22 cells in I-M. All had 9 II.

27 » » II-M. All appeared normal, but the distributions were generally impossible to check owing to the fixation not being quite satisfactory.

Thus it is evident that meiotic disturbances are very common in the intraspecific hybrids. 12 combinations out of 14 selected at random showed abnormalities despite the fact that in many cases the examination could only be rather superficial. The irregularities found consist in incomplete pairing in I-M and, in consequence of this, the occurrence of univalents in the subsequent divisions; also in the existence of inversion bridges and multivalents.

The occurrence of univalents is the most common of the phenomena mentioned; it was observed in 7 combinations of the 11 in which I-M was investigated. Of the 4 combinations with apparently complete pairing, 2 had univalents in the later stages of the meiosis. It is probable that more extensive investigations would have brought to light further combinations with defective pairing, since, *e. g.*, *a*86×*a*81, which is the most pollen-sterile (32 % good pollen) of the cytologically examined hybrids, now only had bivalents. It is very likely that poor fertility in this material is caused by structural heterozygosis by reason of minor deficiencies in the homology between the chromosomes of the parent lines (*cf.* below); and it seems most plausible, too, to explain the common occurrence of univalents in *L. amplexicaule* by a somewhat deficient chromosome homology, adversely affecting the pairing in the meiosis, as expounded in greater detail by MÜNTZING (1938) with reference to the analogous phenomenon in intraspecific hybrids in *Gale-*

opsis. As far as *Lamium* is concerned, this explanation is also supported by a circumstance dealt with below.

Next to the occurrence of univalents, inversion bridges represent the most common form of abnormalities. They have been found in 5 different hybrid combinations; and since stages favourable for the detection of inversion bridges were not available in all the combinations examined, or were sparingly represented, it is probable that several others, too, are inversion heterozygotes. *a*8×*a*12 may be indicated as a probable one, in spite of the fact that no bridges from I-A were found between the II-M groups, because *a*8×*a*9 and *a*9×*a*12 were characterized by inversion bridges in as different frequencies as 1,5 % (138 cells) and 38 % (16 cells) of the PMC's, respectively; this shows that they can scarcely be heterozygous for one and the same inversion. Consequently, *a*8 and *a*12 cannot be structurally equal. It must, on the whole, be borne in mind that the inversion bridges observed probably reveal but a few of the inversions for which the hybrids are heterozygous; minor inversions are not necessarily observed at all, particularly when the chromosomes are as small as those of *Lamium*.

Only the hybrids *a*33×*a*8 and *a*33×*a*86 were distinguished by trivalents. I-M was not studied in *a*8×*a*86, but since this combination has 98 % of good pollen, it is probably a question of the same translocation, or translocations, in both the cases examined. The small size of the chromosomes and the quality of the fixation often rendered it impossible to establish the type of the trivalents but, as a rule, they appeared to be chain-shaped, *i. e.* with only 2 chiasmata.

As pointed out above, it is fairly certain that more comprehensive investigations of the hybrids now examined would have revealed still more signs of structural divergencies. Further, it is not possible to explain the strongly reduced fertility of several of the combinations which have been studied from a cytological point of view, merely by referring to the disturbances observed. Even such hybrids as *a*8×*a*81 and *a*86×*a*81, which have been rather carefully analysed, show all too few abnormalities to account for their low pollen fertility. Further sterility-producing factors must be present.

Very careful cytological investigations, preferably of the pachytene pairing, are required to produce direct evidence of those minor inhomologies between the chromosomes which are not made apparent by disturbances in the process of meiosis, except possibly by the occurrence of univalents in the way indicated above. It is impossible to make such investigations on *Lamium*. On the basis of numerous studies on hybrids

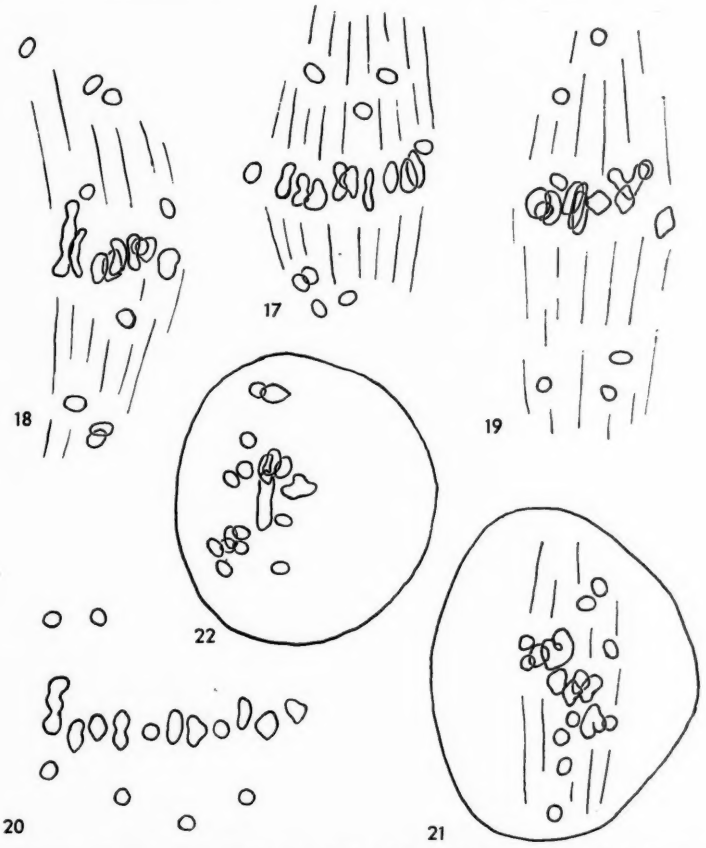
between species, races, lines, *etc.*, these are nowadays considered to be very often characterized by inhomologies of that kind, which are injurious to the fertility. STEBBINS (1945, 1950), among others, has reviewed these matters and given the term »cryptic structural hybridity» to the most minute differences, which are too small materially to influence the pairing at meiosis.

Apart from the actual meiotic disturbances observed and the frequent non-correspondence between their frequency and the degree of fertility reduction, there is a circumstance already touched upon which indicates occurrence of further structural divergences between the lines of *L. amplexicaule*, too. Thus, it was found that hybrids between *L. hybridum* and various lines of *L. amplexicaule* are characterized by greater structural divergences at the meiosis than the intraspecific hybrids of *L. amplexicaule* themselves. This requires a more detailed account which should properly be included in a forthcoming paper on species hybrids in *Lamium* but which must be presented in this connection because of its importance for the understanding of the nuclear conditions in *L. amplexicaule*.

b. Hybrids between *L. amplexicaule* and *L. hybridum*.

Hybrids of this kind have previously been cytologically studied by JÖRGENSEN (1927), who only found 9 II+9 I in I-M and supposed that the chromosome paring mainly takes place between one of the genomes of *L. hybridum* and the genome of *L. amplexicaule*. As shown by later investigations, *L. hybridum* is an allopolyploid; one of its genomes probably comes from *L. purpureum*, while the origin of the other one is unknown (BERNSTRÖM, unpubl.). Possibly it originates from *L. bifidum* ($2n=18$) from South-East Europe, or some closely related species. In appearance, *L. bifidum* is well separated from *L. amplexicaule*.

As will be shown later on in this paper, the bivalent pairing at I-M is very regular in *L. hybridum*, multivalents having never been found. Attention should also be directed to a fact which has previously been reported (BERNSTRÖM, 1950 a) and to which we will revert below, *viz.* that hybrids between several pure lines of *L. hybridum*, which in themselves possess good fertility, never gave any signs of reduced fertility, neither in the pollen nor in the seed-setting. This applies, *inter alia*, to hybrids between the lines *h*18×*h*19 and *h*18×*h*36. No indications of



Figs. 17—22. Chromosome pairing in I-M of hybrids between *L. hybridum* and *L. amplexicaule*. — Figs. 17—19, *h*19×*a*8; Fig. 17, 9 II+9 I; Fig. 18, 1 III+7 II+10 I; Fig. 19, 1 III+8 II+8 I. — Figs. 20—22, *h*18×*a*33; Fig. 20, 1 III+8 II+8 I; separately drawn; Fig. 21, 1 IV+1 III+5 II+10 I; Fig. 22. 1 IV+1 III+4 II+12 I, in early metaphase. — ×3000.

anything that could possibly suggest the existence of chromosome-structural differences between the *L. hybridum* lines have thus been traced.

Studies on I-M of hybrids between *h*19 and *a*8 confirmed that the metaphase groups mostly contain 9 II+9 I, or 8 II+11 I. It was found, however, that also trivalents occur, and — what is of great value for the study of the structural variation within *L. amplexicaule* — it further

TABLE 7. *Frequencies of configurations and univalents in I-M of triploid F_1 hybrids between L. hybridum and L. amplexicaule.*

Hybrids, fixation numbers and slide numbers / Number per cell: IV, III, II, I	Time of fixation	10 7	1 8	9 9	2 6 9	1 7 9	1 5 10	1 7 10	2 5 11	1 6 11	8 11	1 4 12	1 6 12	7 13	1 5 14	4 15	1 6 15	4 16	5 17	Total of cells	% of cells with multivalents
*h*19 × *a*8	10/9																				
L 362 c	1943		3	36				2			17			6						64	8
*h*18 × *a*33																					
L 1730 A 1	16/7		2				2	7	1		5									17	71
» 3	1946			1				2			1		1							5	(60)
» D 1			4	2		1	1	3	2		2	1	6	1	1					24	79
Total			6	3		1	3	12	3		8	1	7	1	1					46	74
*h*36 × *a*8																					
L 3249 h	24/7	1	2	21							16		1	2					1	44	7
» i	1948			8				1			3			1			2			15	7
» k		1		11				2			10			5			1			30	7
Total		2	2	40				3			29		1	8			3		1	89	7
*h*36 × *a*9																					
L 3251 f bud 2	24/7	1	5	16				1		1	8			1			2			35	20
» » 5	1948		4	8				2			8			3			1			26	23
L 3252 f			2	7				1	1		4			1						16	25
Total		1	11	31				4	1	1	20			5			3			77	22
*h*36 × *a*33																					
L 3254 h	24/7		3	5	1		1	3	2		4		3	4		1	3			30	47
» i	1948		1	2	1			4			6		3	3	2		1	2		25	52
Total			4	7	2		1	7	2		10		6	7	2	1	4	2		55	49

turned out, on investigating I-M in *h*18×*a*33 as well, that there is a pronounced difference in the pairing behaviour between the two kinds of hybrids, I-M being far more irregular in hybrids with *a*33, with a higher frequency of trivalents, and occasionally with quadrivalents, too. 8 % of the cells of *h*19×*a*8 contained multivalents, the corresponding figure for *h*18×*a*33 being 74 % (Figs. 17—22).

This led to an investigation of I-M in *a*8×*a*33, the result of which has been presented above and which confirmed the suspicion that this hybrid must be a translocation heterozygote. Its multivalent frequency was, however, only one trivalent in about 9 % of the cells, while a higher figure could be expected. *h*19×*a*8 and *h*18×*a*33 having been fixed in different years and different seasons, the chromosome pairing in I-M was therefore studied also in hybrids between the same line of *L. hybridum*, *h*36, and *a*8 and *a*33, respectively. The fixations were made in the same way and at the same time. With a view to obtaining further light in the matter, hybrids between *h*36 and *a*9 were also included in this investigation.

The results of all the examinations are presented in Table 7. It will be seen from this that there exists a great difference in the frequency of multivalents between *L. hybridum*×*a*8 and *L. hybridum*×*a*33, even if both lines of *L. amplexicaule* are combined with the same line of *L. hybridum* and both hybrid combinations are fixed at the same time. However, the difference between these hybrids is not as great as that between the hybrids first examined. While there is the same frequency of cells with multivalents in *h*19×*a*8 and *h*36×*a*8, *viz.* 8 % and 7 %, respectively, the corresponding figure for *h*18×*a*33 is 74%, but for *h*36×*a*33 only 49 %. χ^2 for the difference in distribution of cells with and without multivalents in the two last-mentioned hybrid combinations is $6{,}45$ ($0{,}02 > P > 0{,}01$); the difference is rather significant.

It is impossible, on the basis of the material at hand, to determine the reason for this difference; it may, of course, have been caused by mere chance. In hybrids between *L. hybridum* and tetraploid *L. purpureum*, it is highly probable that the chiasma frequency is strongly influenced by temperature (BERNSTRÖM, unpubl.), and similar conditions have many times been reported in different material (*cf.*, *e. g.*, FAGERLIND, 1937). In this particular case, the local temperature was actually lower the day before the fixation of *h*36×*a*33 than on the day before the fixation of *h*18×*a*33; in the former case the maximum and minimum temperatures were $19{,}8^\circ$ C and $11{,}7^\circ$ C, respectively, and in the latter $21{,}5^\circ$ C and $15{,}6^\circ$ C. Since the relevant temperature must be

that prevailing at the very time of the prophase, and since the fixations were made in the mornings, the temperatures of the particular interest are those of the days preceding the fixations. It is hard to believe, however, that the difference in chiasma frequency between *h*18×*a*33 and *h*36×*a*33 can be explained merely by a difference in temperature of about 4° C. A difference in temperature can possibly have been a contributory factor, supported perhaps by other external causes, *e. g.*, an occasional difference in the supply of water.

The essential fact remains, however, that hybrids between *a*8 and two different lines of *L. hybridum* are characterized by a considerably lower frequency of multivalents in I-M than are two hybrid combinations between *a*33 and *L. hybridum*. The difference is highly significant; χ^2 for the difference in the distribution of cells with and without multivalents between the hybrids *h*36×*a*8 and *h*36×*a*33 is 34,51 ($P < 0{,}001$). It is evident that this difference between the hybrids of *a*8 and *a*33, respectively, is caused by chromosome-structural differences between the two lines, causing different kinds of pairing with the related genome in *L. hybridum*. As pointed out above, this genome possibly corresponds to that of *L. bifidum*, though perhaps structurally changed in connection with the genesis of *L. hybridum*. Unfortunately, it has not been possible to study any hybrids between *L. bifidum* and *L. amplexicaule*, but preliminary investigations of hexaploid hybrids between *h*19 and *a*9, the chromosome number of which had been doubled by treatment with colchicine, showed that the pairing at I-M is predominantly characterized by bivalent formation (BERNSTRÖM, unpubl.). The average number of configurations and univalents per cell in a plant with 54 chromosomes was 0,3 quadrivalents, 1,0 trivalents, 23,9 bivalents, and 1,8 univalents. The number of trivalents per cell varied from 0—3, of bivalents from 19—27, and of univalents from 0—7. Closely resembling conditions were found in plants with 52 chromosomes.

The predominant formation of bivalents and the corresponding slight rate of multivalents probably imply that the chromosome pairing in the hexaploids is largely homogenetic (WADDINGTON; quoted from STEBBINS, 1947), and that the two genomes from *L. hybridum* and *L. amplexicaule*, which mainly pair in bivalents in the triploid hybrids, are not sufficiently homologous to cause any considerable formation of multivalents in the hexaploids. This would mean that the formation of bivalents in the triploid hybrids on one hand and in the corresponding hexaploids on the other, is similar to that, *e. g.*, of *Primula floribunda*×

P. verticillata where bivalent pairing (of the heterogenetic type), weaker than that of the parent lines, is superseded in the tetraploids by a similarly predominant formation of bivalents which, however, probably occurs between chromosomes of the same parent species (*i. e.* homogenetic associations; STEBBINS, 1950).

Thus, there is reason to suppose that considerable structural differences exist between the genomes which pair in the triploid hybrids. Now, it will be seen from Table 7 that the hybrids between *L. hybridum* and *a*8 never have more than one multivalent per cell. On the other hand, 2 multivalents are formed in 9—15 % of the cells in hybrids between *L. hybridum* and *a*33. The cause of this difference is obviously the same as that which gives to the hybrids with *a*33 a much higher frequency of multivalents per cell than to those with *a*8; *cf.* above, p. 205. The explanation probably is that *a*8 and *a*33 differ in at least two and perhaps still more segmental interchanges, which by pairing with the partially homologous genome of *L. hybridum* get an opportunity to appear just by reason of this genome being different, structurally, from those of *a*8 and *a*33. As regards *h*36×*a*9, 22 % of its PMC's have a multivalent, while the corresponding figure of *h*36×*a*8 is about 7 %. χ^2 for the difference between these hybrid combinations in the distribution of cells with and without multivalents is $8{,}_{14}$ ($0{,}_{01} > P > 0{,}_{001}$), and the significance of this difference is emphasized by the occurrence in *h*36×*a*9 of a cell with two multivalents, which has never been observed in *h*36×*a*8. Evidently, at least one important structural difference must exist between *a*8 and *a*9, most probably consisting in a segmental interchange.

On account of the very regular bivalent formation in *L. hybridum* and the complete non-appearance of aberrant plants resulting from occasional heterogenetic associations (*cf.* below), conjugations are not likely to occur to any considerable extent between chromosomes from *L. amplexicaule* and that genome of *L. hybridum* which is non-homologous to that of *L. amplexicaule*, even though the occurrence of occasional cells with 10 II+7 I might suggest such a thing. The observations of the pairing behaviour of the hexaploid hybrids makes it probable, too, that structural differences between the *L. amplexicaule* lines are made to appear by means of conjugation at least to a quite predominant degree with the homologous genome of *L. hybridum*. The structural differences, with which we are now concerned, are probably rather complicated, and it is not possible, on the basis of the results in Table 7, to give any precise explanation of the nature of the differ-

TABLE 8. *Morphological development of pure lines of L. amplexicaule and their F_1 hybrids.* (*Cf. the text.*)

1. Lines, cross combinations and generations	2. Height of stem				3. Number of internodes			4. Length of leaves				5. Breadth of leaves		
	$\bar{x}$	in % of the parents (*cf.* text)	in % of the highest parent line	*n*	$\bar{x}$	in % of the parents (*cf.* text)	*n*	$\bar{x}$	in % of the parents (*cf.* text)	in % of the largest parent line	*n*	$\bar{x}$	in % of the parents (*cf.* text)	*n*
1944—1														
*a*8	39,4			14	8,79		14	30,3			24	38,3		24
*a*9	50,2			21	10,00		21	35,1			42	35,9		42
*a*10	48,6			14	8,93		14	27,4			19	35,9		19
*a*8 × *a*9	47,1	105	94	14	*10,43*	111	14	33,1	101	94	25	34,2	92	25
*a*8 × *a*10	40,8	93	84	10	*9,20*	104	10	28,2	98	93	17	31,5	85	17
*a*9 × *a*10	48,4	98	96	7	9,60	101	5	29,7	95	85	10	32,3	90	10
1944—2														
*a*8	36,5			11	9,91		11	53,8			27	46,7		27
*a*9	47,1			11	11,36		11	48,0			21	47,3		21
*a*10	39,1			10	10,56		9	42,1			11	36,1		11
*a*32	38,8			11	10,64		11	38,9			21	38,4		21
*a*33	41,3			14	11,29		14	22,8			28	27,6		28
*a*8 × *a*9	47,0	112	100	8	*11,50*	108	8	61,0	120	113	16	51,5	110	16
*a*8 × *a*10	*44,3*	117	113	7	*11,43*	112	7	62,6	130	116	9	50,9	123	9
*a*8 × *a*32	*43,9*	116	113	12	10,60	103	10	49,0	106	91	20	47,8	112	20
*a*8 × *a*33	*42,1*	108	102	14	*11,86*	112	14	35,6	93	66	27	33,5	90	27
*a*9 × *a*10	44,3	103	94	6	11,00	100	6	50,8	113	106	9	44,3	106	9
1949														
*a*8	69,6			10	11,80		10	47,4			18	66,4		18
*a*9	75,7			13	13,08		13	38,2			21	53,2		21
*a*10	82,0			13	12,92		13	31,4			7	46,0		7
*a*12	84,4			11	11,36		11	43,0			26	64,3		26
*a*13	71,3			12	11,33		12	40,7			23	58,5		23
*a*32	59,3			12	10,50		12	24,5			22	34,9		22
*a*33	60,6			12	11,58		12	34,1			22	43,1		22
*a*72	74,6			7	—		—	—			—	—		—
*a*78	67,8			12	12,67		12	41,4			24	53,0		24
*a*86	55,3			8	12,38		8	42,8			16	57,8		16
*a*88	68,8			12	12,50		12	43,9			22	63,9		22
*a*106	74,6			5	—		—	—			—	—		—
*a*108	85,4			5	—		—	—			—	—		—
*a*109	48,3			6	—		—	—			—	—		—
*a*8 × *a*9	74,9	103	99	12	12,17	98	12	47,8	112	101	21	60,0	100	21
								54,4	114	108	22	*69,1*	106	22

*a*106	74,6			5	—		12	43,9			22	63,9		22	
*a*108	85,4			5	—		—	—			—	—		—	
*a*109	48,3			6	—		—	—			—	—		—	

*a*8 × *a*9	74,9	103	99	12	12,17	98	12	*47,8*	112	101	21	60,0	100	21	
*a*8 × *a*12	83,2	108	99	11	10,91	94	11	*51,4*	114	108	22	*69,1*	106	22	
*a*8 × *a*13	*78,8*	112	111	12	11,33	98	12	*55,4*	126	117	26	*69,2*	111	26	
*a*8 × *a*32	*71,7*	111	103	6	11,00	99	6	41,4	115	87	13	57,2	113	13	
*a*8 × *a*33	*75,7*	116	109	11	11,45	98	11	*55,1*	135	116	22	62,7	114	22	
*a*8 × *a*78	*74,8*	109	107	13	11,38	93	13	*48,3*	109	102	26	*67,5*	113	26	
*a*8 × *a*86	*75,5*	121	108	6	11,83	98	6	*56,6*	125	119	16	*72,5*	117	16	
*a*8 × *a*88	*86,8*	125	125	13	*12,54*	103	13	*63,4*	139	134	28	*80,5*	123	28	
*a*9 × *a*10	77,4	98	94	5	11,80	91	5	*40,4*	116	106	10	50,2	101	10	
*a*9 × *a*12	83,6	104	99	14	11,57	95	14	*51,9*	128	121	27	69,1	118	27	
*a*9 × *a*13	*83,2*	113	110	12	12,33	101	12	*52,1*	132	128	23	*68,6*	123	23	
*a*9 × *a*32	68,5	101	90	8	10,88	92	8	37,4	119	98	11	51,7	117	11	
*a*9 × *a*33	*75,8*	111	100	15	12,47	101	15	*57,0*	157	149	30	*66,8*	139	30	
*a*9 × *a*78	73,2	102	97	13	11,85	92	13	46,8	118	113	25	*62,1*	117	25	
*a*9 × *a*86	*78,7*	120	104	12	12,83	101	12	*52,2*	129	122	24	*63,5*	114	24	
*a*9 × *a*88	*83,5*	115	110	4	12,75	100	4	*56,6*	138	129	8	*72,6*	124	8	
*a*10 × *a*12	*86,7*	104	103	9	12,22	101	9	*49,2*	132	114	18	*70,1*	127	18	
*a*10 × *a*13	*89,2*	116	109	9	*13,00*	107	9	*48,3*	134	119	17	*62,6*	120	17	
*a*10 × *a*32	60,7	86	74	12	10,75	92	12	24,2	86	77	20	32,8	81	20	
*a*10 × *a*33	76,7	108	94	9	12,11	99	9	*53,4*	163	157	18	*65,4*	146	18	
*a*10 × *a*78	78,9	105	96	8	*13,00*	102	8	*46,1*	127	111	16	*63,2*	128	16	
*a*10 × *a*86	80,8	118	99	9	12,77	101	9	*45,6*	123	107	18	*58,5*	113	18	
*a*10 × *a*88	89,0	118	109	7	*13,00*	102	7	*56,2*	149	128	14	*72,1*	131	14	
*a*12 × *a*13	*91,3*	117	108	13	*12,15*	107	13	*53,2*	127	124	26	*77,2*	126	26	
*a*12 × *a*32	*84,8*	118	100	11	*11,64*	106	11	*45,5*	135	106	22	64,2	129	22	
*a*12 × *a*33	77,1	106	91	15	11,40	99	15	*48,0*	124	112	30	63,8	119	30	
*a*12 × *a*78	*87,4*	115	104	13	*13,15*	109	13	*49,2*	117	114	24	*70,2*	120	24	
*a*12 × *a*86	78,8	113	93	13	12,08	102	13	*51,5*	120	120	27	*75,0*	123	27	
*a*13 × *a*32	*82,0*	126	115	9	*11,66*	107	9	*45,7*	140	112	18	*64,1*	137	18	
*a*13 × *a*33	*79,0*	120	111	11	*13,09*	114	11	*50,9*	136	125	22	*66,8*	131	22	
*a*13 × *a*78	*85,3*	123	120	14	13,43	112	14	*48,4*	118	117	26	*68,6*	123	26	
*a*13 × *a*86	*79,3*	125	111	14	*13,21*	111	14	*47,6*	114	111	26	*65,3*	112	26	
*a*13 × *a*88	*82,3*	117	115	10	*12,60*	106	10	*49,0*	116	112	20	*66,0*	108	20	
*a*32 × *a*33	*69,2*	115	114	10	*12,10*	110	10	*39,8*	136	117	20	*47,4*	122	20	
*a*32 × *a*78	*70,3*	111	104	10	11,20	97	10	39,5	120	95	20	*54,3*	123	20	
*a*32 × *a*86	*61,9*	108	104	9	11,33	99	9	40,1	119	94	18	52,8	114	18	
*a*33 × *a*78	62,4	97	92	13	11,54	95	13	37,2	98	90	26	44,5	93	26	
*a*33 × *a*86	57,4	99	95	13	11,69	98	13	36,4	95	85	26	44,0	87	26	
*a*33 × *a*88	62,7	97	91	9	12,00	100	9	33,2	85	76	18	44,8	84	18	
*a*78 × *a*86	56,2	91	83	12	11,33	90	12	41,0	97	96	22	55,2	100	22	
*a*78 × *a*88	*73,2*	107	106	9	12,44	99	9	*46,2*	108	105	18	59,4	102	18	
*a*8 × *a*72	64,2	89	86	9	—		—	—			—	—		—	
*a*8 × *a*106	66,1	92	89	8	—		—	—			—	—		—	
*a*8 × *a*108	74,3	96	87	10	—		—	—			—	—		—	
*a*8 × *a*109	*71,5*	121	103	8	—		—	—			—	—		—	

ences between the three kinds of hybrids. The essential thing is that differences between the *L. amplexicaule* lines as regards the occurrence of structural divergences, and possibly also as regards the distribution of structural recombinations between the chromosomes or their arrangement within the chromosomes, *etc.*, have been noticed, or have become more evident, through pairing with the one genome of *L. hybridum*, owing to the fact that this genome is more deviating of structure from either of the chromosome sets of the *L. amplexicaule* lines, than these from one another.

As mentioned above, the meiotic irregularities in hybrids between *a*8 and *a*9 were thus restricted to a very sparse occurrence of univalents and inversion bridges, in themselves indicating inconsiderable structural differences between the lines; no sign of any segmental interchange heterozygosis was noticed. A trivalent was observed in 9 % of the PMC's in hybrids between *a*33 and *a*8, as well as 3, or at least 2, inversion bridges. Consequently, this hybrid combination seemed to be heterozygous for one translocation only, while the configurations in the hybrids with *L. hybridum* indicate the existence of at least two.

C. MORPHOLOGY.

Various measurements were made on the diploid pure lines, as well as on their autotetraploid sister lines, with a view to ascertaining the influence of a doubling of the chromosome number on the morphology and, to some extent, on the rate of growth. When the diploid interlinear hybrids were included in the experiments, these too were measured, in order to establish whether heterosis can appear in this species, which is strictly autogamous but morphologically variable. The other species cytogenetically investigated were measured in approximately the same manner; otherwise, it was not the purpose to examine, in the intraspecific hybrids, the inheritance of morphological characteristics.

The measurements were made only after the plants had ceased growing and their seed-setting had been investigated. Unfortunately, as a result of the short flowering period and the early withering of the plant material, occasional plants, or parts of plants, were often useless at the time of measuring. This accounts, as a rule, for the frequent discrepancies in Table 8 and analogous tables between the number of plants studied, and the measures of their various organs, *etc.* The results of the measurements are presented in Table 8, the headings of which refer to the following measures:

Col. 2 The height of stem from the ground to the uppermost pair of small leaflets which terminates the inflorescence.

» 3 The number of internodes of the stem, including those of the inflorescence.

» 4 The length of leaf of the lowest pair of leaves in the inflorescence. These are generally the largest and best develepod leaves of the plant. Since the petioles of the leaves under discussion are so short and broad that they may, in most cases, just as well be described as part of the laminae, the length of leaf has been taken as being the distance from the tip of the leaf to the stem.

» 5 The breadth of the leaves dealt with in col. 4. By breadth is meant the greatest measure at right angles to the length of the leaf.

Since no clear differences were found between reciprocal hybrids (*cf.* p. 178), the values pertaining to these were added throughout. All 3 generations of *L. amplexicaule* included in Table 8 were cultivated in greenhouse.

Table 8 shows that, in the great majority of cases, the height of the stem and the dimensions of the leaves were greater in the hybrids than the average of the parent lines. This tendency was not evident in 1944—1 but predominated in 1944—2 and 1949. Thus, the height of all the 5 hybrid combinations in 1944—2 exceeded the average of the parent lines, while in 1949 36 of the hybrids were taller and 9 lower than the parents. This distribution, instead of the 1 : 1 ratio which might have been expected, corresponds to a χ^2 of $16{,}20$ ($P < 0{,}001$). As regards the measures of the leaves, the predominance of the hybrids was even greater in 1949, 36 of the 41 hybrid combinations investigated having a greater length of leaf, and 35 a greater breadth of leaf, while 2 showed the same measures as their parents.

These highly significant trends towards greater dimensions of height and leaves than the average of the respective parent lines, were in most cases of such an order that the hybrids exceeded the measures of even the largest parent line in question; in other words, heterosis was at hand in these cases, in the meaning applied to that term by SHULL (1948). In Table 8, the mean values of such cases are in italics. Thus, as far as the height of stem is concerned, 26 of the hybrids in 1949 were taller than the tallest parent line, while 19 were lower. This distribution, instead of 1 : 1, is not significant which, however, is still

TABLE 9. *Time of flowering of pure lines of L. amplexicaule and their F_1 hybrids. (Cf. the text.)*

Lines and hybrids	1944—2			1945—2		
	$\bar{x}$	diff.	*n*	$\bar{x}$	diff.	*n*
*a*8	8,5		12	6,1		11
*a*9	11,4		9	8,2		12
*a*10	5,0		9	5,7		12
*a*12				11,0		10
*a*13				15,1		11
*a*32	4,6		13	3,2		12
*a*33	36,0		14			
*a*8 × *a*9	6,6	—3,4	13			
*a*8 × *a*10	2,9	—3,9	14			
*a*8 × *a*12				2,7	—5,9	9
*a*8 × *a*32	2,5	—4,1	14			
*a*8 × *a*33	20,5	—1,8	14			
*a*9 × *a*10	5,2	—3,0	6			
*a*9 × *a*12				7,2	—2,4	5
*a*9 × *a*13				10,6	—1,1	8
*a*9 × *a*32				2,2	—3,5	12
*a*12 × *a*13				8,4	—4,7	5

the case as regards the length of leaf, with 32 instances against 9 ($\chi^2=12{,}9$; $P<0{,}001$), and, to some extent, also as regards the breadth of leaf, the distribution being here 24 against 14 ($\chi^2=4{,}12$; $0{,}05>P>0{,}02$).

These results, obtained by measurements, were confirmed by visual observations of the plant material. Both in 1944—2 and in 1949, the hybrids, from an early stage right on to maturity, were clearly of more sturdy growth, more evenly grown and richly branched out, and taller than the pure lines. The rate of development could not be studied in 1949 because of the extent of the material, but in 1944—2 and 1945—2, when 5 different hybrid combinations were investigated (*cf.* Table 4), although for lack of time not measured, the beginning of the flowering of each plant was recorded. For this end, the material was reviewed every second day. Starting with the day when the first flowers appeared in the plant material, the average day for the beginning of flowering has been computed for each line and hybrid combination. The results are summarized in Table 9. The abbreviation »diff.» in the table stands for the difference between F_1 mean value and the average of the parent lines. The mean values of those hybrid combinations which were earlier

than the earliest of their parent lines, are in italics. In 1944—2, the 5 hybrid combinations thus began flowering 2—4 days earlier than the parent lines; 3 combinations were earlier than any of the parent lines. Similarly, in 1945—2, 4 of the hybrids were earlier than the earliest parent line, and the 5th hybrid combination was earlier than the average of the parents. The probability that all the hybrids of both 1944—2 and 1945—2 would begin flowering earlier than the average of their respective parent lines, is only $1 : 1024 \left(i.\ e.\ \frac{1}{2^5 \times 2^5} \right)$; consequently, the intraspecific *L. amplexicaule* hybrids were also characterized by a significant tendency towards earlier flowering than that average for the parent lines.

The number of internodes in the hybrids differed from the other characteristics investigated in its relation to the values of the pure lines. The number of hybrids in 1949 with a number of internodes exceeding that of the average of the parents was 19, as compared with 20 having a lower number, and 2 with the same average number; thus, no tendency towards heterosis could be traced in this generation, while this was clearly the case with both the 1944 generations.

Summing up, it may be said that in some of the characteristics measured — and perhaps in the most important ones (*cf.* below) — the hybrid combinations showed a strong tendency throughout to exceed the average size of their parent lines, and that this tendency was often strong enough for the hybrids to exceed even the best developed parent line, implying real heterosis. The behaviour of the hybrids cannot be explained as a result of external conditions; these were the same for the pure lines and the hybrids, although the latter should properly have been placed between the parent lines and not beside them. Limited space, however, did not admit of any doubling of the number of individuals in the pure lines. In 1944—1 there was scarcely any tendency of heterosis, although the arrangement of the plant material was analogous to that of 1944—2 and 1949.

Heterosis, and tendencies in this direction, also manifested itself during well-defined stages of the plant development. It commenced with the beginning of flowering which, in the hybrids, took place after about $^9/_{10}$ of the time required by the pure lines. Flowering begins while the plants are still fairly small. About a weak after the beginning of flowering, when the branches have hardly yet begun to develop, the lowest leaves of the inflorescence reach their full development; after that, they cannot grow further (*cf.* BOYSEN JENSEN, 1943). Finally, the

height of stem and the number of internodes are reached as the last manifestations measured of the capacity of growth.

In order to get an idea of the relation between the size of the various morphological characteristics of the hybrids, the correlation coefficients were computed for the relations, in the hybrids of 1949, between height of stem and number of internodes, height of stem and length of leaf, length of leaf and number of internodes, as well as length and breadth of leaf. In these computations, the values of the hybrids in relation to the arithmetical mean of their parent lines were used. The correlation coefficient, r, for the relation between height of stem and number of internodes is $+0{,}82$ ($t=8{,}9$). For the correlation between height of stem and length of leaf, r is $+0{,}60$ ($t=4{,}7$). The correlation coefficient for length of leaf and number of internodes is $+0{,}24$ ($t=1{,}5$). Finally, there is a correlation coefficient of $+0{,}90$ ($t=12{,}6$) for the relation between length and breadth of leaf.

The correlation values show, on the whole, what could be expected from experience. Heterosis, and tendencies to heterosis, generally affects the entire plant, and not only one or a few features, as pointed out by EAST (1936) and others. Among the features mentioned by EAST as typically influenced by heterosis are, sure enough, the stem, the size of leaf and the higher rate of growth, particularly in the early stages. It is somewhat surprising that the number of internodes did not show the same consistent tendency towards heterosis as did the other features measured; it is, however, perhaps not so much in the nature of heterosis to bring about an increase in the number of parts, as in the size of the parts. There seems to be a parallel to the absence of heterosis in the number of internodes, in the fact pointed out by EAST, that the number of leaves on the stem of tobacco plants do not increase in F_1 hybrids (EAST, 1936). In a similar way, other characteristics, too, will remain unchanged, contrary to expectation.

The correlation coefficient of the greatest interest for the investigations is that between the height of plant and length of leaf, showing, as it does, that those hybrids which were already in an early stage of their development distinguished by the greatest superiority, relatively speaking, to the average of their parent lines in one character, presented, when full-grown, on the whole a corresponding superiority also in another character. This indicates, roughly speaking, that certain hybrid combinations may have been characterized throughout by stronger heterosis, or tendency towards heterosis, than others. This is what could be expected from experience.

The existence of heterosis, and a tendency towards heterosis, in intraspecific hybrids of an autogamous species like *L. amplexicaule*, is certainly of interest in itself; but the reason why this matter has been dealt with at such length in the foregoing is particularly that the present material may, to some extent, throw fresh light on the question of a possible correlation between hybrid vigour and partial sterility. MÜNTZING showed, in a paper from 1945, that intraspecific hybrids of *Galeopsis Tetrahit* are often characterized by heterosis, as measured on the height of stem. Out of 26 hybrid combinations, cultivated in different years, 16 were taller than the tallest parent line, while the other were taller than the average of the parent lines. Partial sterility, as estimated by the percentage of good pollen grains, was very common among these hybrids, and it was interesting to find that there existed a significant negative correlation between the degree of pollen fertility and the degree of heterosis, or tendency to heterosis. Thus, the most pronounced degree of heterosis was found in the most sterile hybrid combinations. MÜNTZING considers the most probable cause of this phenomenon to be the existence of a correlation betwen the degree of genetic diversity and the degree of structural chromosome differentiation between the lines, the latter being, in all probability, the cause of the partial sterility.

From mainly new hybrid combinations within *Galeopsis Tetrahit* and *G. bifida*, HAGBERG obtained results which corresponded well with MÜNTZING's (HAGBERG, 1948).

As far as *L. amplexicaule* is concerned, a corresponding investigation was made on the hybrids of 1949. The correlation coefficient between pollen fertility and height of plant, expressed in percentage of the mean value of the parents, is — 0,08, the correlation thus being non-existent. Excluding the hybrids with *a*33, which occupy a somewhat isolated position in the correlation table, a correlation coefficient of — 0,14 is obtained ($t=0{,}8$; $P > 0{,}4$).

If, instead, the height of plant of the hybrids is expressed in percentage of the tallest parent line (*cf.* Table 8), which is perhaps a more proper way of estimating the degree of heterosis, the correlation coefficient between height of plant and pollen fertility is — 0,25 ($t=1{,}5$; $0{,}2 > P > 0{,}1$). Hybrids with *a*33 are not included here; if included, r will be — 0,15.

Consequently, there is not much except the minus sign in front of the correlation coefficients to indicate a correspondence, as regards the conditions investigated, between *L. amplexicaule* and *Galeopsis*. A sug-

gestion of a similarity appears if the height of plant is correlated with the seed-setting instead of the pollen fertility. Thus, in the pure *L. amplexicaule* material of 1949, the coefficient for the correlation between seed-setting and height of plant, expressed in percentage of the mean value of the parents, is $-0{,}30$ ($t=1{,}8$; $0{,}1 > P > 0{,}05$). If, however, the seed-setting is correlated with the height of plant, in percentage of the tallest parent line, a correlation coefficient of only $-0{,}15$ is obtained.

As will be seen, the material investigated lends but the slightest support to MÜNTZING's supposition, based on results from *Galeopsis*, that there may exist a positive correlation between the degree of genetic diversity and the degree of structural chromosome differentiation. Various circumstances which are, possibly, able to produce different results in the two experimental materials, may however be pointed at. Thus, the degree of heterosis is rather slight in *L. amplexicaule*, whereas it appears to be more pronounced in *G. Tetrahit* (MÜNTZING, 1945 a; HAGBERG, 1948). Most probably, the capacity — or facility — for increased vigour is different in different plant genera, and a greater capacity of that kind will evidently increase a correlation like that examined here. Further, it is a well-known fact, which has appeared also in the hybrids within *L. amplexicaule*, that the degree of heterosis often varies with the seasonal variations and the cultural conditions (*cf.*, *e. g.*, GUSTAFSSON, NYBOM and v. WETTSTEIN, 1950; MALINOWSKI, 1950); MALINOWSKI also draws attention to the fact that hybrid vigour in *Phaseolus vulgaris* is always more pronounced when the material is cultivated in the field than in pots. Should the same apply to *Lamium*, too, stronger and more differentiated manifestations of heterosis between the hybrid combinations might have been obtained by other methods of cultivation, resulting in other coefficients for the correlations investigated.

It is also possible that the extent of the chromosome structural divergencies between the lines of *L. amplexicaule* is less than that of *G. Tetrahit*. The visible irregularities of I-M in intraspecific hybrids of *G. Tetrahit* — no other stages were analysed — appear to be more extensive than those in *L. amplexicaule*, but the fact that the former species is a tetraploid one renders comparisons difficult. Nor may the extent of the chromosome structural variation in the respective species be deduced from the degree of fertility reduction in the interlinear hybrids, a considerable part of this feature, which appears in a diploid like *L. amplexicaule*, being probably suppressed by the tetraploid nature of *G. Tetrahit*.

The fact that *G. Tetrahit* is by no means as strongly autogamous as *L. amplexicaule*, may also imply that chromosome structural divergencies are more frequent in the former than in the latter (*cf.* MÜNTZING, 1930, pp. 188—189; 1938, Table 1 and p. 163). A frequent consequence of allogamy is the presence of a widespread structural heterozygosis within the population which should be more easily accumulated and borne by a tetraploid species than by a diploid one. The morphological variation in *G. Tetrahit* is largely a consequence of continuous hybridization with *G. bifida*, resulting also in a perpetual formation of new chromosome structural types.

On the other hand, we have no direct knowledge regarding the origin of the structural variability of *L. amplexicaule*. For the present, and for divers reasons, we must assume that this, too, was brought about by interspecific hybridization and has been further developed by occasional interlinear cross-pollination, which however on account of the pronounced autogamy of the species is far more sporadic than in *G. Tetrahit*.

MÜNTZING (1945 a) also discusses the question whether the correlation between heterosis and partial sterility may be of a physiological nature, by the partially pollen-sterile hybrids showing also a somewhat reduced seed-setting; as the latter makes smaller demands on the yielding capacity of the plants, more is left for their vegetative growth. The suggestion is rejected as rather improbable, because the plants probably stop growing before the seeds reach maturity.

Some fresh arguments against such a hypothesis emerge from the investigations of *L. amplexicaule*. Thus, the correlation between height of plant and seed-setting (*cf.* above) is pretty weak. Yet, *L. amplexicaule* should be very well suited for an investigation of this nature, since its seed matures already 12—13 days after the flowering, far more than half the total amount of seeds having therefore matured and fallen off by the time the height growth ceases. The other reason, and perhaps the most important one, is the fact that measurements of the size of the leaves and, to some extent, also of the commencement of flowering have shown heterosis to have clearly developed long before the earliest seeds have matured. It takes something like a week, after the measured leaves have attained their final size, before seed maturity begins in the earliest flowers, and even then the majority of flowers are in the embryonic stages of development.

(To be continued in a forthcoming issue.)

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HETEROSIS IN F_1 COMBINATIONS IN GALEOPSIS. II

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VIII. VARIABILITY.

GUSTAFSSON (1946) has found a tendency towards greater individual variation between barley plants heterozygous for lethal genes than between normal homozygotes. GRANHALL (1946), however, did not observe a similar increase in variation among the heterozygotes in his wheat material. Some data about the variation in the present material have already been given in the sub-sections on flowering time and cell size. In this section a further discussion of the variability in the material is presented.

The variation in a material of homo- and heterozygotes can be studied from at least three different viewpoints. *First*, one may ask whether there is a difference between homo- and heterozygotes in regard to the variation within the individual in characters such as cell size. *Secondly*, one may study the variation between isogenous plants within the biotypes; this is the type of variation which GUSTAFSSON (*l. c.*) called plasticity and found to be greater between heterozygotes than between homozygotes in his and in ROBERTSON's material. *Thirdly*, there is the type of variation to which special interest has been devoted in this study, *viz.*, the variation between the heterozygous biotypes in comparison with the variation between the corresponding parental pure lines.

GUSTAFSSON (*l. c.*) used the coefficient of variation as a measure of variability. The use of this coefficient presumes a direct proportionality between mean and standard deviation. Such a proportionality is not a general rule in nature, and the use of the coefficient implies more or less of an approximation and is not suitable for all types of variation. The present author has tried to use statistically more adequate methods for a comparison in regard to variability between materials with widely different mean values.

The variability within heterozygous plants, as compared with that within homozygous ones, has been studied in some F_1:s and the corresponding parents with regard to the size of the stomata and the pollen

grains. The analyses of variance of the data are given in Tables 13, 15 and 16, and are discussed in Part I, sub-section VII: 7, pp. 72—74. The variation in size of the stomata is significantly greater in the F_1 T—B× T—C than in the parent lines, which might indicate some kind of a disturbance of the balance in the hybrid plants.

The variation between isogenous plants has been studied in the same material. F_1 is intermediate in this respect in regard to the size of the stomata, and also in regard to the size of the pollen grains in the combination T—C×T—F, while the combination T—B×T—C shows a slightly significant tendency towards a greater inter-plant variation in the heterozygote.

GUSTAFSSON (*l. c.*) writes: »It appears as if the heterozygous variability, so striking in young stages, would gradually narrow down». The

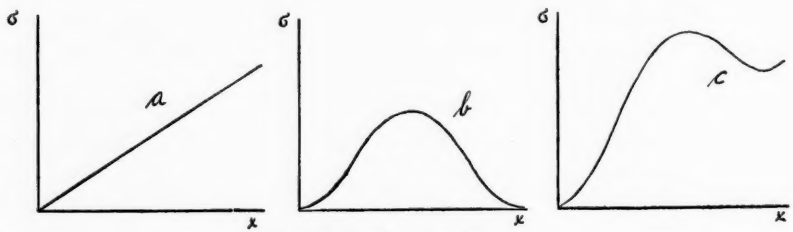


Fig. 28. Explanation in text.

present author has studied the variability in plant height at different stages of development. The method used has been as follows: diagrams have been made showing the relation between mean plant height and standard deviation at the different instances of measuring, the mean values taken as x and the standard deviations as y. The points in the diagrams have been marked with figures, 1 indicating the values of the first measurement, 2 the second, *etc.*; all points with the same figure indicate measurements made on one and the same day. In reality the diagram is three-dimensional.

The shape of the curve obtained is influenced by three factors: (1) The increase in variability with an increase in the mean; the factor which is considered in calculating the coefficient of variation, or, in suitable cases, the regression of standard variation upon mean. (2) The increase or decrease in variability with an increase or decrease in the growth rate, causing a reduction in variability towards the end of the period of growth. (3) The eventually greater variability in the hetero-

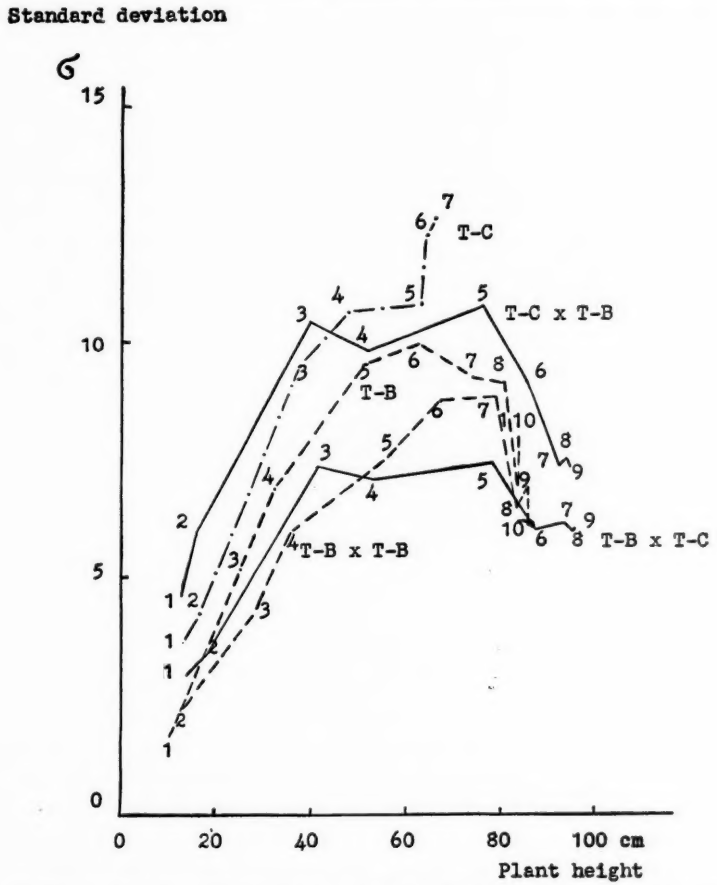


Fig. 29. The relation between plant height and standard deviation during the period of growth in the cross T—B × T—C, 1946.

zygotes, the subject of the present study. The two first factors separately would cause diagrams of the types shown in Figs. 28 *a* and *b*, respectively; together they will result in a curve of the type shown in Fig. 28 *c*. A study of the actual diagrams obtained will make it possible to find out the eventual effect of the third factor.

Diagrams of the type described are given in Figs. 29 and 30 for some of the combinations studied, as representative examples. It should

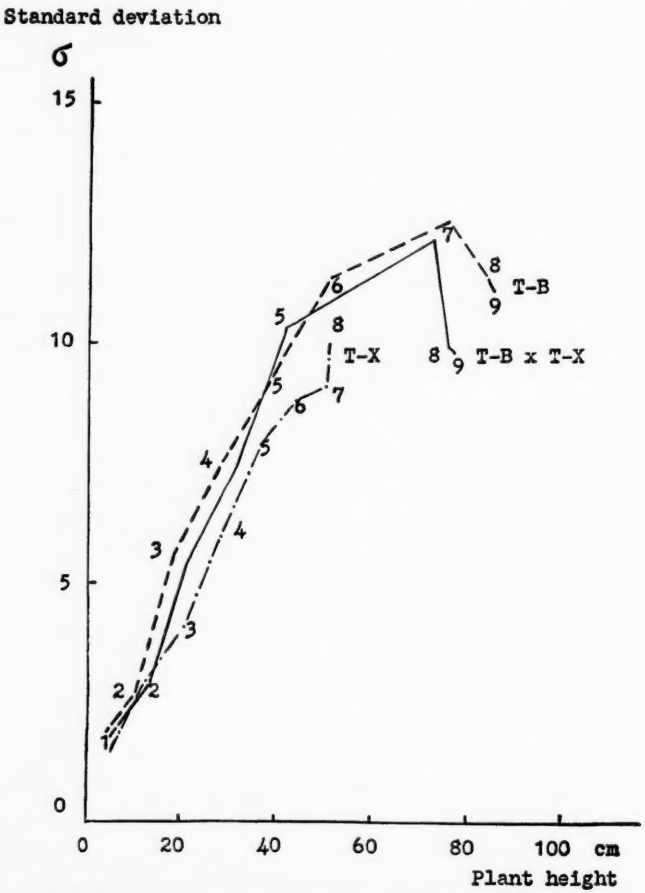


Fig. 30. The relation between plant height and standard deviation during the period of growth in the cross T—B × T—X, 1947.

be observed that in a year with an extremely dry summer, as in 1947, the curve is less influenced by factor 2 than in a more normal year, as for example in 1946. The curves give no indication whatever that F_1 should be more variable than the homozygous parent lines.

A study of the relation between mean value and standard deviation in dry weight results in a correlation diagram of the type presented in Fig. 31, which represents the material of 1946. The triangles indicate values for the parent lines, the circles those for the F_1:s. Values belong-

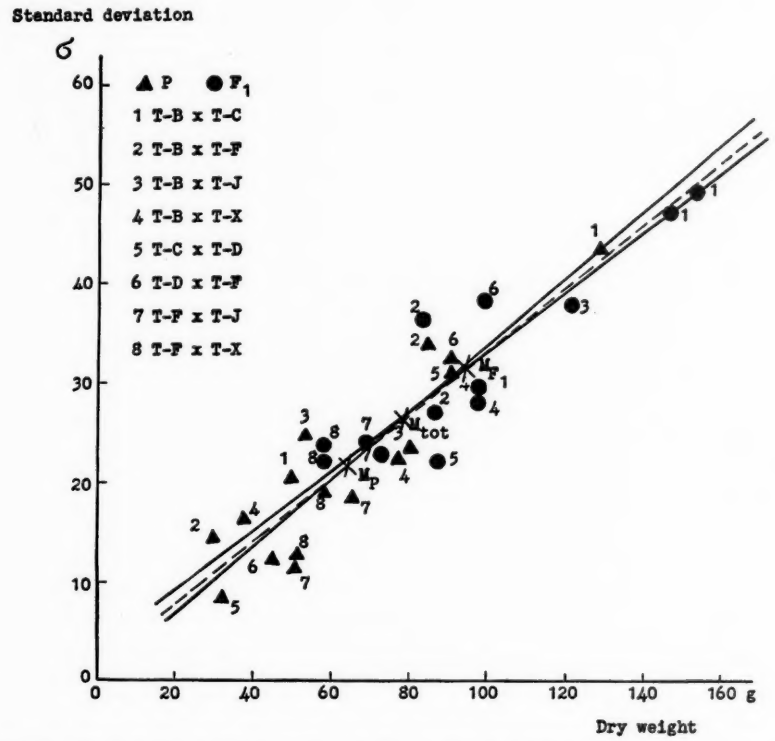


Fig. 31. The relation between means of dry weight for different biotypes and the corresponding values of standard deviation, 1946.

ing together are marked with the same figure. There are two sets of values, those representing F_1 and those representing the parents. For each of these as for the total material the coefficients of regression are determined. The result of the analysis of covariance is given in Table 19.

As seen from the table, there is no significant difference between the two regression lines, nor do the means for F_1 (M_{F_1}) and for the parents (M_P) deviate significantly from the average regression line. All data may, therefore, be considered as belonging to the same statistical population, which has a regression coefficient of $+0{,}316 \pm 0{,}0329$. The average regression line is drawn in Fig. 31. Above this line there are 8 points representing P biotypes and 5 representing F_1 biotypes. Below the line there are 8 values of each group. Thus, there is no general

TABLE 19. *Analysis of covariance of the regression standard deviation upon dry weight of F_1 and P.*

Cause of variation	DF.	Sum of squares	Variance	Rows	Quotient
1. Total variation in standard deviation ..	28	3210,96	—	—	—
2. Total regression part	1	2758,68	2758,68	2/3	164,70***
3. Total deviation from the average regression line	27	452,28	16,75		
4. Deviation of the means from the average regression line	1	0,57	0,57	4/5	< 1
5. Deviation from average regression lines through the means	26	451,71	17,37		
6. Difference between the regression lines of F_1 and P	1	13,90	13,90	6/7	< 1
7. Deviation from the separate regression lines of F_1 and P	25	437,81	17,51		

indication that the variation between individuals should be greater in F_1 than in the parent lines, as regards dry weight of ripe plants.

In the data concerning the time of flowering there is no correlation between mean and standard deviation. An analysis of covariance shows a weak and hardly significant coefficient of correlation, and the coefficient for the regression of the standard deviation upon the mean is only $+0{,}04$. In this material the standard deviation may be directly used in a comparison as regards variability between different biotypes. Table 20 presents data concerning the variability in flowering time for some of the combinations studied. The table gives the differences in standard deviation between, on the one hand, F_1, and, on the other, the parents, the most variable one as well as the mean of both. The variability of F_1 is somewhat less than that of the most variable parent, and somewhat greater than the average, but none of the differences is significant, and in regard to flowering time there is no indication of a greater variability in F_1 than in the homozygous parent lines.

After having dealt with variation within plants and between isogenous plants it remains to discuss the variability between biotypes among heterozygotes and homozygotes. As shown in Part I, sub-section about flowering time (VII: 5), the F_1:s varied significantly less from their common mean than did the averages between the two parental lines in a pair. In section VII: 9 it was pointed out that a similar tendency can be observed in regard to »general rhythm of development», although in this case it was impossible to demonstrate the phenomenon by exact figures as in the case of the flowering time.

These studies of the variability of heterozygotes and homozygotes may be summarized as follows: In one combination F_1 has shown greater variability within the plant in regard to the size of the stomata. The variation between plants within a biotype is of the same order of size in the heterozygous F_1 as in the homozygous parent lines. The variability between the F_1 biotypes is less than between the corresponding parental biotypes.

As to the intra-plant variability, GOODSPEED (1912) showed an increase in variability in the size of flowers in *Nicotiana* hybrids, which may have occurred within or between plants. According to SHULL (1914), the reason for this increase in variation is that »a highly heterotic plant, for instance, because of its unusual vigor may develop branches from buds which in a weaker plant would remain dormant. The foliar or floral organs borne by such branches may differ from those on the branches which usually develop». This explanation can hardly be valid for the case of increased intra-plant variation in the size of stomata reported here. A tentative explanation is that it is due to a disturbed genic balance.

TABLE 20. *Variation in time for the beginning of flowering. Differences between the standard deviation of F_1 and, first, the most variable parent line (P_{max}), secondly, the mean of both parent lines (P_{mean}). Means for 1945—1947.*

Combinations	F_1—P_{max}	F_1—P_{mean}
T—B × T—C	— 0,51	+ 0,06
× T—D	— 1,38	— 1,24
× T—F	— 1,88	— 0,69
× T—I	+ 0,01	+ 0,53
× T—J	— 1,09	— 0,80
× T—X	+ 0,14	+ 0,21
T—C × T—D	— 0,48	+ 0,05
× T—F	— 0,42	— 0,21
T—D × T—F	— 0,68	— 0,25
T—F × T—I	+ 1,71	+ 1,97
× T—J	— 0,33	+ 0,08
× T—X	+ 0,19	+ 0,30
T—B × B—E	— 2,07	— 0,88
× B—F	+ 1,75	+ 1,79
T—F × B—E	— 1,39	+ 0,07
× B—F	— 0,17	+ 0,09
B—E × B—F	— 0,61	+ 0,62
M ± m	— 0,424 ± 0,233	+ 0,100 ± 0,204
M/m	1,68[9]	< 1

In contrast to GUSTAFSSON's (1946) cases of an increased »plasticity» in plants heterozygous for one or two lethals, SHULL (1914) is of the opinion that an F_1 plant showing heterosis is less susceptible to variations in the environment. SHULL does not present any data in support of his theory. Data from crosses without heterosis were published by JOHANNSEN (1907): »Resuming these experiments, it may be said that the fluctuations as to weight and dimensions in the pure lines were not less than in their hybrids». HAYES (1912) obtained similar results in *Nicotiana*: »The F_1 generation is no more variable than the parents, the variability of F_1 being found slightly greater than the average of the parents in six cases and less in five cases». The opinion of SHULL is further confirmed by the data summarized in this section, as also by the fact mentioned at the end of section VII: 5 (Part I), that the variation in flowering time between the years is the same in F_1 and parents.

GUSTAFSSON's very interesting data from his own material and that of ROBERTSON contain the only cases so far reported which indicate a higher degree of variability or plasticity in heterozygotes in comparison with homozygotes. His observation, however, that the increase in variability is most marked in the early stages leads to the hypothetical explanation that it depends upon an increase in the growth rate in these stages, *i. e.*, it is dependent upon the factor listed as number 2 above.

IX. THE RELATION BETWEEN FERTILITY AND THE DEGREE OF HETEROSIS IN F_1.

MÜNTZING (1945) found a probable relation between the fertility and the degree of heterosis in F_1, measuring the latter as the difference between the plant height of the hybrid and the average height of the two parents. Later, HAGBERG (1948) has confirmed the results of MÜNTZING, using the dry weight of the plant as a measure of its production and the relation between F_1 and the superior parent as a measure of the degree of heterosis.

MÜNTZING's data in the paper of 1945 have been recalculated according to the definition of heterosis accepted by the present author. Thus, the plant height of F_1 has been determined in relation to the tallest of the parents. In this way a non-significant positive correlation between fertility and heterosis is obtained, the coefficient being $r = +0{,}30$, instead of $r = -0{,}50$, as found by MÜNTZING.

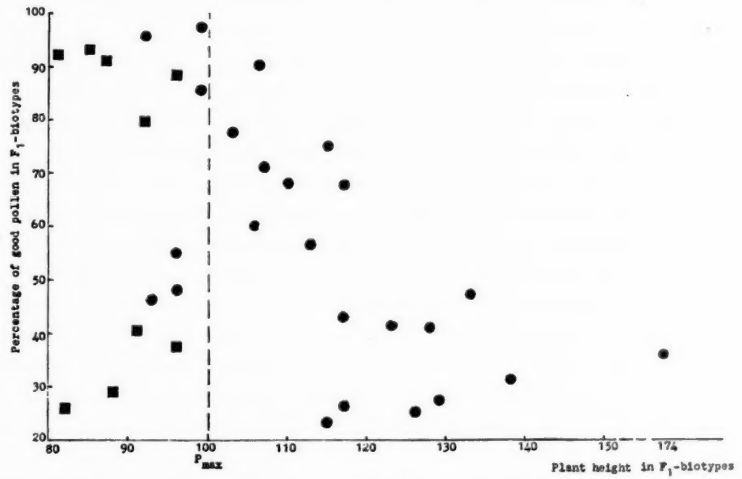


Fig. 32. The relation between the relative plant height of the F_1 biotypes (the tallest of the parent lines, $P_{max}=100$) and their pollen fertility. 1950. Square points indicate combinations where one of the parents is T—G.

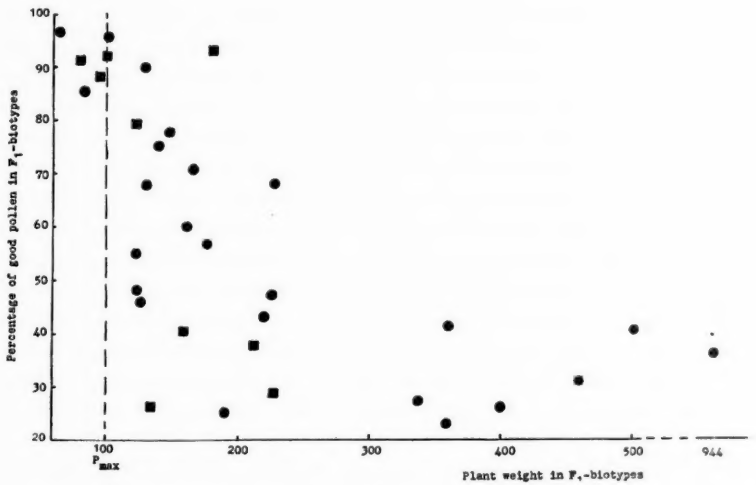


Fig. 33. The relation between the relative plant weight of the F_1 biotypes ($P_{max}=100$) and their pollen fertility. 1950. Square points indicate combinations where one of the parents is T—G.

Figs. 32 and 33 present the distribution of the pairs of values for the heterozygous biotypes in the material of 1950. They concern the correlation between the fertility in F_1 and the degree of heterosis in height and in dry weight. For plant height the correlation is rather good but without strong statistical significance; $r = -0{,}44$, $t = 2{,}70^{*}$, $0{,}02 > P > 0{,}01$. The values for crosses within *bifida* are not included, but there is about the same correlation in this material, too.

As in MÜNTZING's (*l. c.*), there is also in the present material a somewhat stronger negative correlation between fertility and heterosis in plant height, if the latter is determined in relation to the mean of the parents, than when the tallest parent is used. For the material of 1950 one obtains $r = -0{,}45$, $t = 2{,}81^{**}$ and for that of the years 1945—1947 the values are: parental mean as basis, $r = -0{,}49$, $t = 2{,}18^{*}$; tallest parent as basis, $r = -0{,}44$, $t = 1{,}88^{0}$, $0{,}1 > P > 0{,}05$. These groups of material investigated in different years are to a certain degree complementary and, thus, the correlation is rather well confirmed.

There is a strong negative correlation between the height of the tallest parent and the degree of heterosis measured with the height of this parent as 100, but the regression is not a straight line. There is also a strong negative correlation between parental height and heterosis when the mean value of the parents is used in the calculations. A cross between two low lines usually shows a high degree of relative heterosis; a cross between two tall lines usually gives no heterosis at all in height but often in weight. Even here the inter-biotypic variation is smaller in F_1 than among the parent lines, but in this case the distribution curve of the former is not concentrated around the mean of all the material but rather tends towards the positive end of the parental curve.

If the diagram over the correlation between fertility and heterosis in height is studied more in detail, it is observed that one and the same genome usually reacts in a typical manner in all combinations. This is most evident in the case of T—G, all combinations of which are found to the left in the diagram. The fertility of the species hybrids is about 35 %, that of the intra-specific combinations 80—90 % good pollen. The relations between the values for the combinations of T—G and, *e. g.*, the *bifida* lines, agree fairly well with the average relations between the values for these *bifida* lines in other combinations. Obviously, the agreement is not complete. Such a detailed analysis, as pointed out by HAGBERG (1948), also shows that the relation between fertility and degree of heterosis should not be treated merely as a simple, uncomplicated phenomenon.

The diagram in Fig. 33 shows the relation between fertility and the degree of heterosis in dry weight ($P_{max}=100$). The coefficient of correlation is $r=-0{,}52$, $t=3{,}85$, $P<0{,}001$. This value from 1950 shows good agreement with the results published in 1948 (HAGBERG, *l. c.*). The corresponding values of the material investigated in the years 1945—1947 are: parental mean as basis, $r=-0{,}80$, $t=5{,}17^{***}$; heaviest parent as basis, $r=-0{,}82$, $t=5{,}48^{***}$.

The relation between fertility and degree of heterosis is, as already mentioned, influenced by two factors. One is the unavoidable experimental error caused by the shedding of the seeds. It may be pointed out, however, that the F_1 T—G×B—E has a heterosis value of about 135, the F_1 T—I×B—C one of about 400. The degree of sterility and the shedding are about the same in both cases and evidently shedding alone cannot be responsible for the high degree of heterosis observed in several of the partially sterile combinations.

The second factor influencing the relation is the well-known phenomenon that a sterile plant continues flowering and branching after the normal time for the ending of the growth. In *Galeopsis* the main axis has a limited growth and the plant height is not influenced by the continued growth, which instead consists of the formation of new branches of higher orders. This phenomenon in itself is a result of hybridization and a kind of heterosis, dependent upon the sterility. That it alone cannot explain the observed high degree of heterosis is again shown by a comparison between T—G×B—E and T—I×B—C.

It would be of great interest to measure the effect of heterozygosity which is independent of fertility, and for this purpose growth curves for dry weight would be valuable. As has been stated, it has not been possible to obtain a sufficient number of F_1 plants for the determination of such curves. Nor does it seem to be possible to fix with sufficient accuracy any biologically defined stage before ripening when the dry weight might be determined, free from the influence of shedding and continued growth.

That the relation between fertility and degree of heterosis is not a simple, uncomplicated phenomenon is evident from the fact that the variation in degree of heterosis is greater in the partially sterile group than within the fertile one. This can be easily seen from the diagrams in Figs. 32 and 33, in the case of height as well as weight. The hybrids with the highest degree of heterosis are found among the partially sterile ones, but in this group there are also cases, *e. g.* T—F×T—I, where F_1 is inferior in some characters. This difference in variability is

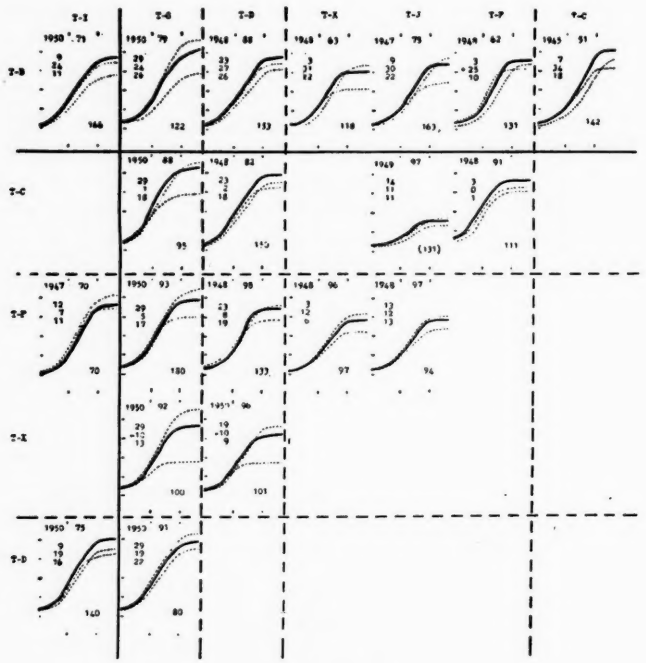


Fig. 34 *a*.

Fig. 34 *a—d*. Growth curves for plant height and some further data from crosses between lines of (a) *Tetrahit*, (b) *bifida*, (c) *Tetrahit* and lines of *bifida*, (d) the diploid species. The continuous curve is the growth curve of F_1. The curves of short dashes are those of the parents. The figure to the left at the top is the year in which the observations are made. Beside the year the pollen fertility of the F_1 is recorded, and below, the day for the beginning of flowering, first, that of the P-line on the top of the diagram, secondly, that of the P-line at the left of the diagram, and last that of the F_1. The figure to the right, below, is the relative dry weight of F_1, when $P_{max} = 100$. (In material from 1949 + indicates > 100, — is < 100.) Vertically the plant height is given as 0, 25, 50, 75, 100 cm, and horizontally the time is given: July 1st and Aug. 1st. The curve starts at June 1st. The parental lines are grouped in the same way as in Figs. 2—5 and in Table 8.

found also in MÜNTZING's (1945) data. If his material is divided into one fertile and one partially sterile group, using 90 % good pollen as class-limit, there are 10 and 14 biotypes, respectively, in the groups. The relation between the variances for relative heterosis within the groups is $6{,}99^{**}$.

The relation between sterility and heterosis is, as pointed out, a

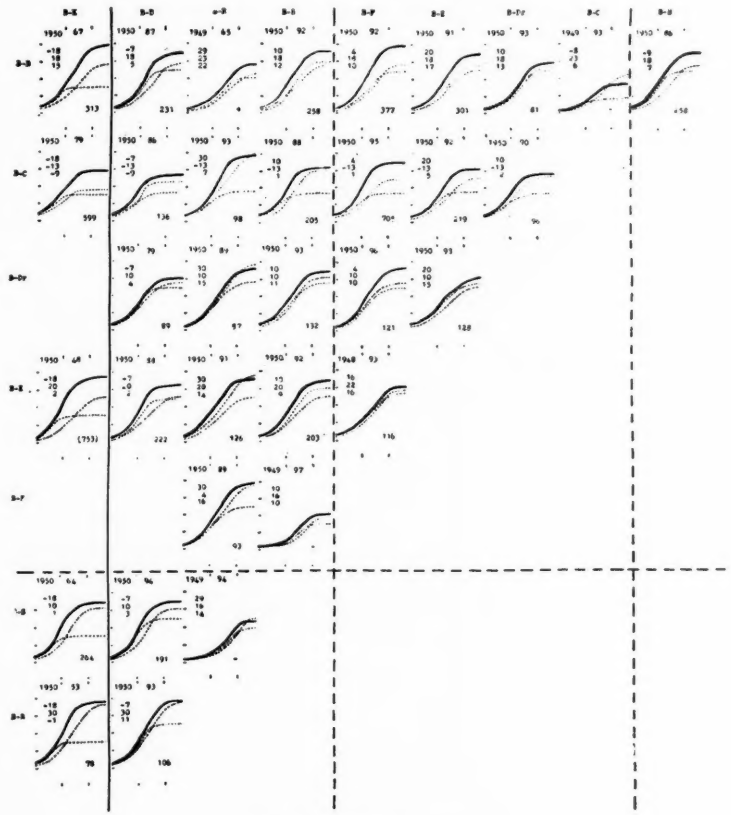


Fig. 34 *b*. (See page 232.)

complex one, and this may at least partly depend upon different causes for the reduction in fertility: (1) This may not be related to a high degree of genotypical difference between the parents, but be caused, for example, by a single segmental interchange (*cf*. ROBERTS, 1942). In such cases heterosis is hardly to be expected, and they will not contribute to the correlation discussed. (2) In most cases where there is a great genotypical difference between the parents, this is probably combined with structural differences in the chromosomes, causing reduced fertility in the hybrids. If an increased heterozygosity results in increased heterosis this would (a) normally result in the observed negative

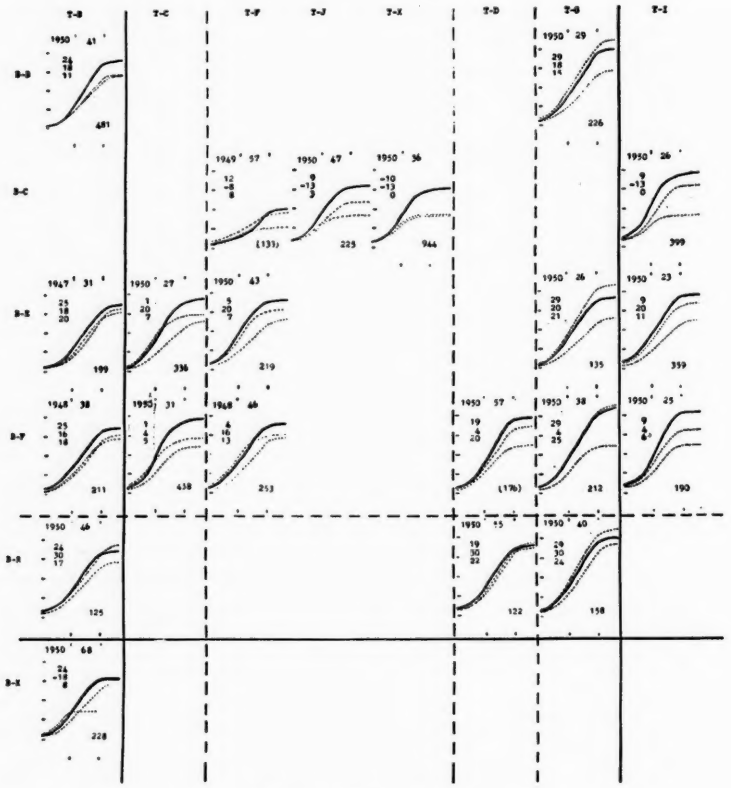


Fig. 34 *c*. (See page 232.)

correlation between fertility and degree of heterosis. It is, however, quite possible that (b) in some cases the combination of genes from two parents may be unfavourable (*cf.* T—F×T—I, Fig. 34 *a*) and the result would be an F_1, breaking the more general relationship.

X. DISCUSSION AND CONCLUSIONS.

WHALEY (1944) points out that the literature on heterosis can be divided into two main groups. One of them deals mainly with the theoretical-genetical explanation of the phenomenon, while the other is more descriptive of its manifestations and attempts to elucidate the direct causal relations. The present study of the F_1 of a number of

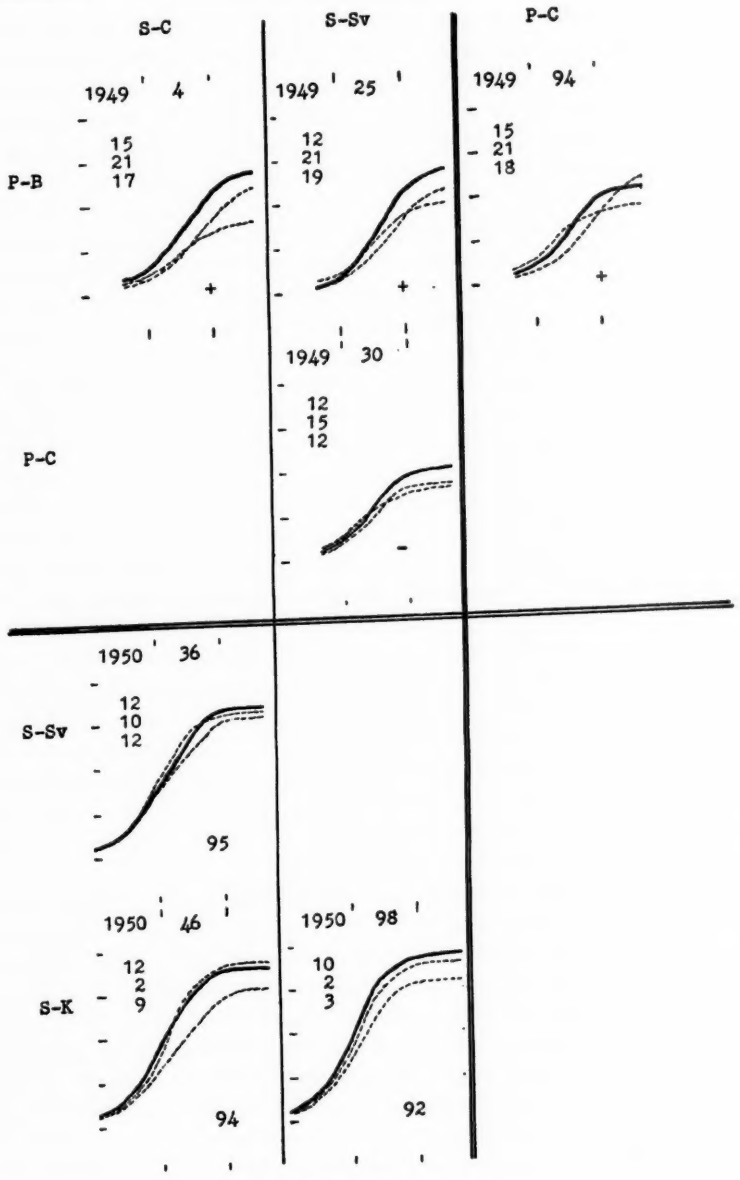


Fig. 34 *d*. (See page 232.)

crosses within *Galeopsis* falls, on the whole, within the latter group. In dealing with the detail problems the author uses JONES' factorial explanation of heterosis as a fundamental basis for the discussions.

Some of the initial problems, mentioned in the introduction, have already been discussed in previous sections and the answers given as far as the material permits; the remaining problems are discussed in this section.

MÜNTZING (1938) searched for a relation between the geographical origin of the *Tetrahit* lines and their grouping into inter-sterile groups. His conclusion was that: »according to the data recorded above, it is evident that the geographical origin is of little or no importance as regards the sterility constitution». The corresponding question in the present study has been: Is there any relation between the difference in geographical origin between the parent lines and the degree of heterosis in the F_1? The answer is as negative as in MÜNTZING's case: the same high degree of heterosis may be obtained upon crossing parent lines from the same locality as in crosses between lines from widely different localities. If the degree of heterosis stands in relation to the genotypical differentiation between the parents it may therefore be concluded that there is no relation between geographical and genetical diversity. The explanation is probably to be found in the mode of migration as well as in the mode of fertilization of the species studied.

Seed of *Galeopsis* is fairly commonly found as a weed in seeds of cultivated plants, especially clover, and the result must be a fairly effective and continuous new introduction into Scandinavia as well as a circulation between different parts of the region. The species of *Galeopsis* here studied are strongly influenced by cultivation, and they are mainly found growing in cultivated areas or on the border-line between such areas and, for example, a wood.

The two species *Tetrahit* and *bifida* are autogamous, and within such species natural selection must act more slowly than in allogamous species. This fact in combination with the steady influx of new material in any given habitat probably explains the lack of differentiation into climatic or edaphic ecotypes observed in this material, and also the lack of relation between geographical and genotypical differentiation, as observed both by MÜNTZING and the present author. There are, however, exceptions to this general lack of differentiation. MÜNTZING, *e. g.*, has described a shore ecotype of *G. bifida*.

In several genera, species with a high chromosome number are more self-fertile than low-chromosomed species. LEVAN (1936) has

shown that self-fertility increases with an increased chromosome number in *Allium*. GUSTAFSSON (1947), in a chapter on polyploidy and its influence on cross-fertilization, gives a review of the literature on this problem.

In the present study it has been shown that seed-setting in *Galeopsis Tetrahit* is somewhat higher after the use of alien pollen than if pollen from the same pure line is used. This indicates some kind of mechanism favouring cross pollination, in spite of the complete self-fertility. This mechanism is, possibly, a remnant of a system of *s*-alleles, which has functioned in the more allogamous, diploid ancestors. Polyploidy partially breaks down such a system of *s*-alleles, and in combination with the occurrence of reduced fertility in many F_1:s this gives a selectional advantage to biotypes suited for selfing, whereby full fertility may be more rapidly attained.

The autogamous reproduction of the species studied excludes any comparisons between the F_1:s here investigated — although they are structural heterozygotes (MÜNTZING, 1938) — and the structural heterozygotes in allogamous genera, such as *Drosophila* (DOBZHANSKY, 1948) and *Trimerotropis* (WHITE, 1945) since the autogamy prevents any effect of eventually selectional advantages for the heterozygotes.

By synthezising *Tetrahit*, MÜNTZING (1930 b) has shown that this species with 32 chromosomes is an allotetraploid derived from the two 16-chromosome species *speciosa* and *pubescens*. MÜNTZING's synthetic *Tetrahit* was, however, not a regular amphidiploid, since some of the s-chromosomes in one of the genomes were replaced by p-chromosomes, which MÜNTZING denotes by giving his synthetic form, AT, the symbol $\frac{\text{p s}}{\text{p r}}$.

MÜNTZING (1938) considers that the existence of intra-specific sterility barriers within *Tetrahit* may be due to spontaneous hybridization between this species and *bifida*: »Thus, though this needs to be verified, there is reason to assume that the multitude of structural types in *G. Tetrahit* is closely connected with the frequent natural hybridization between *Tetrahit* and *bifida*». This may be correct, but this connection is probably of a secondary importance. The present author is of the opinion that the close relationship between the two species, as shown by the frequency of spontaneous crosses as well as by the partially overlapping variation in morphological characters, makes it probable — although not proven — that *bifida* also is an allopolyploid derivative of *speciosa* and *pubescens*.

If such is the case the species complex *Tetrahit+bifida* may have a polyphyletic or a monophyletic origin, the fairly marked difference between the two species giving some preference to the former hypothesis. In both cases the sterility barriers may have originated in different ways. There are sterility barriers within the diploid species (*cf.* Part I, page 70, and MÜNTZING, 1938), probably of the same nature as those within the tetraploids. If the tetraploids have originated several times, the partaking diploid biotypes may have belonged to different inter-sterile groups, and this difference is inherited directly from diploid ancestors to tetraploid. If the tetraploids in nature have been formed only once but in the same complicated manner as in MÜNTZING's experiment, or in a similar one, more than one biotype from one or both of the diploids may have participated, and these may again have belonged to different fertility groups. Even without the latter assumption the fertility groups may be the result of the complicated process of origin of the tetraploid, the cause of sterility being the incomplete homology between the s- and r-genomes in MÜNTZING's notation. Finally, it is possible that the sterility barriers within the tetraploids are the results of accumulations of structural changes that have occurred within a poly- or monophyletic stock.

Quite possibly the sterility barriers within the *Tetrahit+bifida* complex may be caused in more than one of the ways discussed. At any rate, it seems most probable that the fertility groups at least to some extent indicate a grouping after relationship and that the genetic differentiation is greater between groups than within, which again agrees very well with the fact that some of the partially sterile F_1:s show an especially high degree of heterosis.

Of great interest is a comparison between diploids and tetraploids in regard to the degree of heterosis. LUNDQVIST (1947 and unpublished data) has shown that the inbreeding degeneration is less marked in autotetraploid rye than in the diploid. The result of crosses between inbred lines of autotetraploids is, so far, not known. Theoretically, one would not expect a higher degree of heterosis than within the diploids. The allotetraploids, on the other hand, behave, genetically and cytologically, essentially as diploids. They have the chromosomes of both the diploid parents and may, therefore, be heterozygous for an absolutely — but not relatively — greater number of loci than the latter, and it is quite probable that they may show a higher degree of heterosis (*cf.* EAST, 1936). In the limited material here presented the heterosis is on an average more marked in the allotetraploids than in the diploids.

It remains to discuss in which characters and at which stages of development heterosis is most marked. It is shown that in some crosses the F_1 embryo in the ripe seed is superior (*cf.* ASHBY and others). It has also been shown that the superiority of F_1 is often founded upon a rapid growth of the radicula and SPRAGUE (1936) has pointed out that the superiority of the hybrids is founded in the meristematic growth — the cell division.

In a series of papers WHALEY *et al.* (1950), HEIMSCH *et al.* (1950) and RABIDEAU *et al.* (1950) have studied two inbred lines of maize and their hybrid. They state: »In the maize cross ... the hybrid advantage is not apparent in embryo weight but is developed during early seedling growth and is maintained to maturity. It is manifest in greater root and shoot weights, greater total internode length and volume, and greater total leaf length and area ... The larger root system of the hybrids absorbs more radiophosphorus than the root system of the inbreds. The pattern of distribution of radiophosphorus is essentially the same in the inbreds and the hybrid». Their final conclusion is: »The hybrid advantage in radioactive phosphorus absorption is associated with more rapid early development, earlier attainment of maturity, and differences in vascular organization».

The type of heterosis described by the authors quoted is characterized by the increase in meristematic growth in the seedling stage, giving the hybrid an advantage which lasts throughout its growth. Several examples of this type of heterosis are found in the present material, and they are especially common when both parents develop more slowly than the average at this stage, *e. g.*, the cross T—B×B—R.

The causation of heterosis is somewhat different in crosses like T—B×T—C, where F_1 grows about as rapidly as T—C in the seedling stage and later on has the same growth rate as T—B, the later of the parents. In this case F_1 is not superior in growth rate at any definite stage of development to the parent best at that stage, but it obtains a favourable combination between two parental growth rhythms, which results in a superiority in total growth and total production of dry matter, which latter character has been used here as the main measure of heterosis.

The two types of causation are not fundamentally different, since they may both be explained on a factorial-genetical basis and in both cases heterosis is dependent upon the »dominance» relations in the genic basis of different characters. As two extreme types of the varying »mechanism of heterosis» there may be quoted, on the one hand, the

maize case just discussed (transgression heterosis), on the other hand, the well-known case of KEEBLE and PELLEW (1910) where a marked heterosis in plant height in *Pisum* is caused by the combination of two dominant, major factors. The many cases of »combination heterosis» in *Galeopsis*, shown in this paper, may be considered as cases more or less intermediate between these extreme types, thus indicating combination of favourable characters of the parents, resulting in heterosis.

Thus, from the point of view adopted here the »somatic type of heterosis» (GUSTAFSSON, 1951) may be subdivided in the following way:

(1) *Heterosis directly caused by genotypic differences*:

(a) *Combination heterosis*: F_1 combines from the parents two or more traits, each one favourable for the total »vigor», without showing heterosis in any one of them separately; the single traits may be mono- or polygenic, but the »dominance» for each must be favourable.

(b) *Transgression heterosis*: F_1 shows heterosis at some stage of development, perhaps most often at the seedling stage, and this is sufficient to cause a superiority in total »vigor».

(2) *Heterosis indirectly caused by genotypic differences*:

The heterozygote shows a more or less marked sterility, which results in a supernormally long continuation of vegetative growth and, thus, in an increase in dry matter production. Between this type of heterosis and that which is directly caused there exists a deep fundamental difference.

In the *Galeopsis* crosses here presented heterosis is found in regard to plant height and dry matter production. It is most marked in crosses between relatively low parents, less so when the parents are tall, and there is, thus, a relation between the height of the parent lines and the degree of heterosis in F_1. The mode of measuring heterosis used in this paper, *viz.* by the relative value of F_1, if the superior parent is=100, magnifies the mathematical measure of this relation, but it is equally evident, if heterosis is determined as the absolute difference between F_1 and parents. If one or both of the latter are very tall, F_1 rarely reaches the value of the tallest of them in height; in regard to dry matter production, heterosis is somewhat more common even in crosses between lines with high production values.

The relation observed is one example of a more or less general rule, very well known, among others, by plant-breeders: the higher the yield of the existing varieties in a district, the more difficult it is to obtain an increase. It may at least partially be explained by the »interaction of genes» discussed, among others, by RASMUSSON (1933), which implies

that the effect of any one single factor, favouring, *e. g.*, growth in height, decreases with an increase in the number present of factors with a similar effect.

It is by no means a foregone conclusion that great plant height or dry matter production in themselves have a positive selective value, even if it seems probable that, under similar conditions, plants with a more »vigorous» vegetative growth will also have a greater competitive power; at least they must be expected to be superior in this respect to the other extreme, low growing plants with low production.

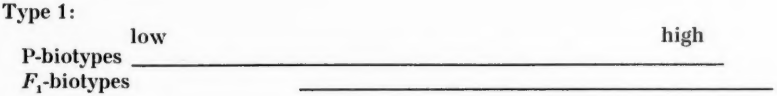
In regard to flowering time, on the other hand, it is impossible to expect, *a priori*, that any of the extremes should have a *general* selective superiority. Of course they have under certain extreme environmental conditions, but not on an average of all the species. In the present material the relation between the parental values and those of F_1 in regard to this character is, in fact, distinctly different from the corresponding relation in regard to height and dry matter.

Two distinct types of »dominance» conditions have been observed in the material:

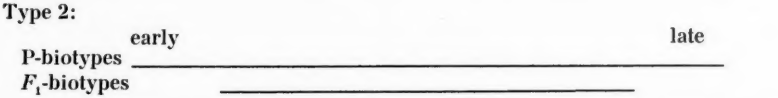
(1) The heterozygote often reaches or surpasses the superior parent and the variation of the whole population of different F_1:s is shifted towards higher values in comparison with the population of the parent lines (type 1 in the diagram below); only exceptional F_1:s (T—F×T—I in the present material) have values as low as that of the inferior parent. This is shown for height and dry matter in the present material.

(2) There is no general tendency for F_1 to have more extreme values than either parent but the variation of the F_1 population is more concentrated around a medium value than is that of the parent lines (type 2 in the diagram below). This is shown for flowering time in the present material.

Type 1:



Type 2:



Having found the distinction between the two types of »dominance» conditions, it is tempting to embark on the following speculative discussion. As pointed out above, the autogamy of the allopolyploid species

of *Galeopsis* is probably acquired rather late during their phylogeny, and for the development of dominance the theory of FISHER (1930) may be valid. According to this theory, dominance is the result of a selection of modifying genes, tending to strengthen the phenotypical effect of an allele whose initial effect results in a selective advantage. On this theory, and accepting JONES' (1917) general explanation of heterosis as a result of dominance as well as MATHER's (1949) opinion that observable potence indicates true dominance, it would be possible to draw the conclusion that great height and great dry matter production on an average have had a positive selective value. On the other hand, extreme earliness or lateness during the phylogeny of the species in question probably has been on an average selectively disadvantageous. It also follows that heterosis is only to be expected in characters where plus variants are selectively superior or have so been in previous phylogenetic stages without now being disadvantageous.

As already stated, the author has so far in his discussions accepted the general theory that heterosis is due to a combination in the heterozygote of a large number of dominant factors, derived from both parents. Even if this should be the main explanation of heterosis, it is quite conceivable that there are contributary causes of greater or less importance. One such contributary cause would be a »single factor heterosis» or superdominance; *i. e.* the relation $Aa > AA > aa$ between the three possible genotypes within a single pair of alleles, a relation which in itself is just as possible as $AA = Aa > aa$ (complete dominance) or $AA > Aa > aa$ (incomplete dominance). The problem of single factor heterosis will be dealt with in a following paper.

Acknowledgement. — The author is indebted to his teachers and colleagues at the Institute of Genetics, Lund, and at the Swedish Seed Association, Svalöf, for valuable help, discussion and criticism. Especially I want to thank Dr. O. TEDIN for many good suggestions and for translating the manuscript. The investigation has been facilitated by financial support from the Nilsson-Ehle Foundation and from the Royal Physiographical Society of Lund.

SUMMARY.

(1) The F_1 of 81 different crosses has been studied, the parents being pure lines within the four species *Galeopsis speciosa, pubescens, Tetrahit,* and *bifida.*

(2) In order to obtain fully comparable material of the parents, artificial crosses have been made within the lines. No difference is found between progenies after selfing and after crosses between plants within the line.

(3) In the fully autogamous *G. Tetrahit* the seed-setting is somewhat better in crosses between lines than in crosses within the line.

(4) In certain combinations a difference between the reciprocals has been observed. If the *Tetrahit* line T—B is used as mother in species crosses with *bifida* the germination of the F_1 seed is retarded, probably because of an excessive amount of some germination-inhibiting substance; seeds of the reciprocal combination germinate rapidly and well.

(5) Heterosis is found in the growth rate at different stages of development, radicula growth, young plants and the time of most intensive growth. On the other hand, final heterosis is often caused by a favourable combination of the growth rhythms of the parents, and in these cases not by dinstinct heterosis at any given stage.

(6) The plant height has been measured at regular intervals, thus giving growth curves for the different combinations as a basis for a study of the occurrence of heterosis.

(7) The time of the opening of the first flower has been determined. The values for the F_1:s vary less around a common mean than do the values for the parents.

(8) Studies of pollen fertility have shown the existence of partial sterility in some crosses within the species *bifida* and *speciosa*, just as earlier found by MÜNTZING in *Tetrahit*, and it has been possible to group the lines also within the first mentioned two species into intrafertile groups.

(9) The sterility barrier between *Tetrahit* and *bifida* varies in intensity with the lines used for the cross.

(10) There is no relation between the size of cells and heterosis.

(11) There is a certain indication of a higher mutation rate in the heterozygotes as compared with the homozygotes.

(12) On comparing heterozygotes and homozygotes as regards variability, these have been subdivided into three groups: (a) variability within plants; in one F_1 studied, the size of the stomata varies more in F_1 plants than in the parents; (b) variation between plants within a biotype; there is no indication in the material of a higher variability of this kind in the heterozygote than in the homozygote; (c) the variability between all the different F_1 biotypes as compared with that between

the corresponding parents has been found to be lower in regard to flowering time.

(13) There is a relation between reduced fertility and a high degree of heterosis, but the relationship is a complex one. The degree of heterosis varies more among the partially sterile F_1:s than among the fully fertile ones. When sterility is correlated with a high degree of genotypical difference it should also be correlated with a high degree of heterosis; if the sterility is caused by a single structural difference, no such correlation with heterosis is to be expected.

(14) No relation between geographical differentiation and degree of heterosis is found in the material. The explanation is sought in the autogamy of the species studied and their mode of migration.

(15) The degree of heterosis is on an average higher in the allotetraploid species than in the diploids.

(16) The phylogeny of the sterility barriers within and between the tetraploid species is discussed.

(17) An attempt is made to subdivide the »somatic heterosis» of GUSTAFSSON into: (1) heterosis directly caused by genic differences and (2) heterosis indirectly caused; sterility of the hybrid leading to continued vegetative growth. Type 1 is again subdivided into (a) combination heterosis and (b) transgression heterosis, and the different »mechanisms of heterosis» are discussed. All these types of somatic heterosis are observed in the present material; *cf.* point 5 of this summary.

(18) Two different types of »dominance» or »potence» are found in different characters in the material, and their bearing upon the selective value of the characters in question is discussed.

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ABSTRACTS

A. LIMA-DE-FARIA: The chromomere size gradient of the chromosomes of rye.

A detailed chromomere analysis of the seven chromosomes of the normal complement of *Secale cereale* revealed, among other things, the existence of a chromomere size gradient in these chromosomes (LIMA-DE-FARIA, 1952). This detailed chromomere analysis includes the study of the number, size and disposition of the chromomeres of each chromosome, thus permitting the construction of a map of each chromosome type.

A study of the maps of the chromosomes of the normal complement of rye reveals the following features:

(1) Every chromosome possesses the same general chromomeric pattern with minor variants.

(2) In all chromosomes of the normal complement the chromomeres are particularly large on both sides of the kinetochore. There is a subsequent general decrease in chromomere size on both sides of the kinetochore towards the chromosome ends, where an abrupt increase in chromomere size takes place in most cases (knob formations). This gradient of chromomere size originates on both sides of the kinetochore, where chromomeres of large size are found, and finishes in chromomeres of very small size at the chromosome ends, or just behind the knob formations when these are present.

(3) This gradient originates on both sides of the kinetochore, yet the decrease in chromomere size that takes place in the arms is not only dependent on the presence of the kinetochore.

(a) Evidence of the apparent control of the chromomere size gradient by the knob formations is found in the deviating pattern of the left arm of chromosome V. The comparison of the left arm of chromosome V with any arm of the same length, as, for instance, the left arm of chromosome VI, shows that although these two arms are approximately of the same length, the rate of decrease in chromomere size is quite different in the left arm of chromosome V from that in the left arm of chromosome VI. An inspection of the chromomeric structure of the left arm of chromosome V shows that the existence of an intercalary knob formation belonging to the nucleolar organizing region is the apparent determining factor of the abrupt fall of the chromomere size in this arm. This can be seen from the fact that the chromomeres decrease in size from the kinetochore to the intercalary knob formation; and from this to the distal knob formation they keep an irregular size with no connection with the gradient that originates at the kinetochore. The intercalary knob formation apparently not only controls the rate of decrease in chromomere size, but also stops the gradient that starts at the kinetochore.

(b) In arms where no knob formation is present, which end in small chromomeres or only in a pair of large chromomeres, as in the right arms of chromosomes III, IV, VI and VII, it can be seen that the rate of decrease in chromomere size is not the same in the different arms. Whereas in the right arm of chromosome IV, which has approximately half the length of the right arm of chromosome VII, the decrease in chromomere size is sufficiently abrupt to result in the presence of small chromomeres at the end of the arm, in the right

arm of chromosome VII this decrease is, on the contrary, much more smooth. This decrease in the right arm of chromosome VII is such that the chromomere size gradient does not finish at the middle of the arm as could be expected if the gradient were independent of arm size, but extends itself throughout the whole arm, finishing exactly at the end of the arm in chromomeres of the smallest possible size (chromomeres that are near the limit of microscopical resolution). The same is true for the right arm of chromosome VI, where the rate of decrease in chromomere size is adapted to the length of the arm in such a way that the gradient of chromomere size, which originates at the kinetochore, finishes at the arm end, and not at some other region of the arm, in chromomeres that are near the limit of microscopical resolution.

Thus, in the chromosomes of the normal complement of rye two distinct phenomena apparently determine the general chromosome pattern: (1) the existence of a chromomere size gradient that originates on both sides of the kinetochore, (2) the regulation of the rate at which this decrease in chromomere size takes place. This regulative process is apparently controlled in the first place by the knob formations and, in their absence, by the arm length.

Three different sources of evidence reveal that the formation of the chromomere size gradient depends primarily on the properties of the kinetochore rather than on the properties of the chromosome arms that exhibit it:

(1) The formation of the chromomere size gradient is independent of the particular genetic constitution of the arms of the chromosome that carries it. The chromomere size gradient is exhibited by *every chromosome* of the normal complement of rye and for this reason cannot easily be considered to depend for its formation on the particular genetic constitution of the arms of each chromosome (*cf.* DARLINGTON, 1937).

(2) The chromomere size gradient is not only independent of the particular genetic constitution of the arms of the chromosome that carries it, but is also a phenomenon independent of the particular genetic constitution of rye, because similar gradients are found in the chromosomes of the normal complement of other plants such as *Agapanthus umbellatus* (*cf.* LIMA-DE-FARIA, 1950).

(3) The chromomere size gradient originates on *both sides of the kinetochore* in every chromosome of the normal complement of *Secale cereale*, *Agapanthus umbellatus* and other species.

The existence of this gradient in the chromosomes of rye and the variation of its shape in relation to knob formation position and arm length, indicate that the size of a chromomere is determined not only by its genetic constitution and nuclear environment, but is also dependent on its position within the chromosome. Experimental evidence of such a phenomenon is found in the results obtained by SCHULTZ (1941) and by KAUFMANN (1948) in *Drosophila*. These authors found that in different types of rearrangements, the nucleic acid content of a disc and the appearance of its chromomeres will depend not only on the general environment provided within the nucleus but also on its position within the chromosome.

The phenomenon of the chromomere size gradient is most easily interpreted by assuming the streaming of a substance from the kinetochore along the chromosome body, which affects the appearance of the chromomeres and in this way partly controls the heteropycnotic appearance of the different

chromosome regions. Heteropycnosis appears as a phenomenon determined not only by the genetic constitution and nuclear environment of a certain chromosome region but also by the position of this same chromosome region within the chromosome body.

LONGLEY (1939) was led to suggest the existence of a chromosome gradient. He found the frequency of knob formations to be a function of the distance of a knob formation building-point from the kinetochore. His supposition is now confirmed by the structural analysis carried out in rye.

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NILS NYBOM: A device for facilitating the paraffin embedding procedure.

At the Institute of Genetics at Lund the vials used for embedding root tips and flower buds in paraffin consist of ordinary watch glasses, about 6 cm in diameter. A pair of forceps is used for arranging the objects radially in small groups near the periphery of the »paraffin cake». Two difficulties are, among others, involved in this procedure. Firstly, it is sometimes difficult to see, *e. g.*, the thin root tips of many materials in the whitish paraffin; and, secondly, it is necessary to interrupt the work every few seconds to warm the forceps in a nearby flame.

The device to be described here may well meet these two difficulties. It consists of a lamp, by which the watch glass is illuminated from below, and a pair of forceps, which is kept warm electrically. The device is depicted in the semi-schematical drawings of Figs. 1—4.

The lamp house is made of a small metal paint-pot with a diameter and a height of 5—6 cm. See Fig. 1. In this pot a little lamp is placed, fed by a transformer (door-bell transformer giving 2 W at 4 V). In series with the lamp a variable resistance of 50 ohms is put in. This lamp gives a very suitable, translucent light making it substantially easier to work even with thin and colourless objects. By means of the rheostat the light intensity is easily adjusted in relation to the room light. The lamp house also forms a suitable support for the watch glass with the melted paraffin.

The electrical forceps, shown in Fig. 2, is made of a piece of steel plate, about 0,8 mm thick. Both tips are made broad, and the broad edges are rolled

up longitudinally. In the tubes thus formed there are inserted two pieces of resistance wire (Fig. 2, *a*) put into contact with the poles of another transformer. When the forceps are brought together the current passes through the resistance wire, whereupon the tips immediately become warm enough to melt the paraffin. In Fig. 3 we see in greater detail how the resistance wire is insulated and fixed in the forceps. A piece of rubber-insulated 6-wired copper cable (Fig. 3, *a*) is soldered to the thin, flexible cable (*b*) and fixed in the metal

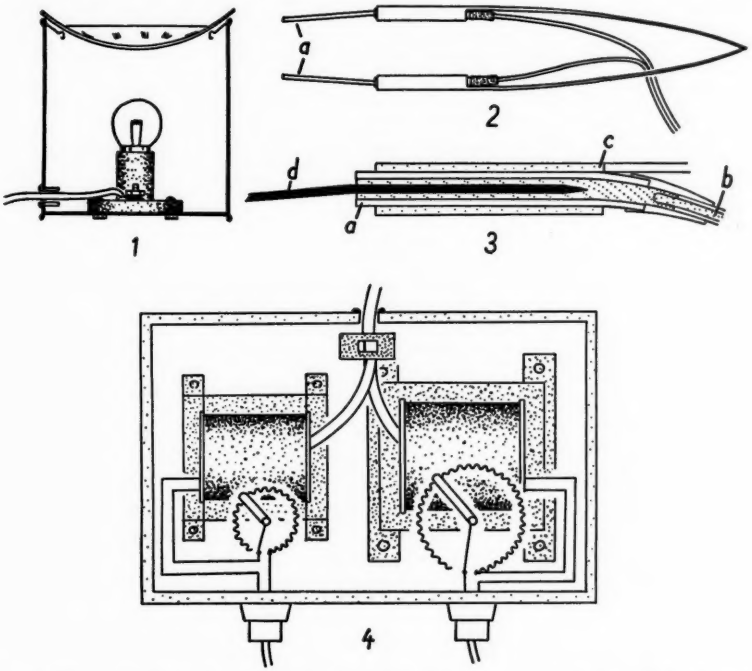

Fig. 1. The embedding lamp. — 2. The electrical forceps. — 3. Detail of the forceps showing the method of attaching the heating wire. — 4. The box containing the transformers and the resistances. — The drawings are reduced according to the following scales: 1, 2,1 ×; 2, 1,7 ×; 3, 0,7 × and 4, 3,5 ×.

plate of the forceps (*c*) by bending this around it. The resistance thread (*d*) is inserted into this first copper cable (*a*). The flexible cable (*b*) leads to the second transformer, which has to be more powerful than the first one.

As the choice of transformer may cause trouble I venture to add that a type of transformer found to have been very suitable is one which has various outputs for 4 up to 24 volts and giving 1 amp. The 4 volt output is used.

In series with the forceps a variable resistance of about 5 ohms is also put in, tolerating more than the lamp resistance, *viz.* about 25 W.

The resistance wire used for the forceps is a chrome-nickel wire (Kanthal), 0,9 mm in diameter and with a specific resistance of 2,2 ohms per metre.

These forceps are very handy to work with as they are always kept warm, though the metal coming into contact with the hand is cool. It is no longer necessary to interrupt the work in order to warm the forceps in a flame.

The two transformers may be placed in a wooden box as indicated in Fig. 4. This box also houses the two rheostats and a switch to the main. Alternating current is, of course, necessary, if transformers are to be used. If there is only direct current available it should be possible to make some arrangement for using resistances instead of transformers.

The device described here has been in almost daily routine use at this institute for several months and has been found to work satisfactorily in facilitating the paraffin embedding procedure.

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